MANUAL OF VITAL FUNCTION TESTING METHODS AND THEIR INTERPRETATION

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TO DR. GEORGE M. KOBER, DEAN OF THE GEORGETOWN MEDICAL SCHOOL, THIS LITTLE BOOK IS DEDICATED, AS A MODEST TOKEN OF APPRECIATION OF HIS UNSELFISH DEVOTION TO OUR SCHOOL AND HOSPITAL

PREFACE TO FIRST EDITION

THERE should always be some valid reason for writing a new book, especially a medical book. My reason for writing, or perhaps to express it more accurately, for compiling this work, is, that the information that it contains is scattered quite broadly through a wide and extensive medical literature, that may not be readily accessible to all, and which has never, so far as I know, been collected together in book form.

Inasmuch as every progressive physician and surgeon at the present day is making more or less frequent use of the different functional tests, to determine the efficiency of vital organs, it occurred to me that to collect them all in one volume, together with the necessary data whereby they might be intelligibly interpreted, might prove to be useful, particularly to the busy practitioner.

The following work, which is an effort to carry out that idea, includes only the tests for so called vital functions, namely those applied to the liver, kidneys, heart, pancreas, and ductless glands. To go beyond this and take up the functional tests of all the organs, such as the eye, ear, nervous system, etc., would be to exceed the legitimate field of true function testing and to encroach upon the well-trodden fields of general semiology and diagnosis.

I hope the book may prove to be useful and convenient to all who are interested in this fascinating and ever-developing field of clinical pathology.

It is a matter of which American physicians may well be proud that the most substantial and brilliant progress in the development of tests of organic function, particularly in reference to the kidneys and liver, has been brought about by the assiduous efforts of some of their own fellow countrymen.

In the chapter on heart I have received valued assistance from my friend and colleague, Dr. Thos. S. Lee.

PREFACE TO SECOND EDITION

A NEW edition of the *Manual of Vital Function Testing Methods* has been called for within a year of the appearance of the first. This is exceedingly gratifying to the author and his colleagues who have helped him in the preparation of the second edition.

The additions made are as follows:

Dr. Thos. S. Lee, Clinical Professor of Medicine in Georgetown, Medical College, for whose assistance in the chapter on heart in the first edition I am under deep obligation, has added to the second edition articles on Sphygmobolometry, Sphygmobolography and Energometry.

Dr. Lester Neuman, Associate in Pathology in the Georgetown Medical College, has made very valuable additions to the second edition which will be found in the chapters on liver and kidney function. These additions include a description of the Van Slyke method of urea determination, Provocative Sulpho-Conjugation as a means of testing hepatic function, Test Meal investigations of kidney function, Ambard's coefficient and McLean's index of urea excretion, Creatinin and uric acid estimations in blood and finally a synopsis of Mosenthal's and Lewis' scale of renal involvement.

The author with the aid of one of his assistants, Dr. Ladd, of the house staff of the Municipal Hospital, has added a short description of functional tests applied to the Vegetative Nervous System, which is appended to the chapter on the Ductless Glands.

It is hoped that these additions will bring the *Manual* up to date and thereby justify a continuance of the very favorable reception which the book has received among those of the medical profession who are interested in vital function testing.

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CHAPTER I

TESTS OF LIVER FUNCTION

GENERAL CONSIDERATIONS

The liver cell represents the whole organ in miniature. If we possessed an adequate knowledge of all the functions of this cell, we would understand completely the functions of the organ.

The functions of the liver cell are numerous and each individual cell performs its quantitative quota of all these functions. The liver is provided with two evacuating channels, one internal, by way of the blood (hepatic vein), the other external, by way of the biliary passages. It receives blood by way of the portal vein from the digestive tract and its related organs, and discharges into the duodenum its more or less complex excretion.

Regarded as a center of elaboration, the liver cell has several important functions, chief of which are: 1. ureagenetic, 2. glycogenetic, 3. lipasic, 4. antitoxic or cytopexic, 5. sanguinopoietic, 6. thermic, 7. ferric. The above mentioned functions are spoken of as endocrinous. The liver has, however, an exocrinous function which by some is regarded as the most characteristic, namely, the excretion of bile by way of the biliary passages.

Tests of hepatic function are directed towards the determination of the integrity of one or the other of these phases of activity.

There is no organ whose functional and clinical examination is fraught with more difficulties than the liver. Slight functional disturbances of the organ are attended by very uncertain symptomatology. When severe organic lesions exist, such as acute yellow atrophy, cirrhosis, abscess, cancer, etc., modifications in the size of the liver, as well as its consistence, together with symptoms of portal hypertension (ascites) and of biliary obstruction (icterus), offer a combination of objective evidence which makes the diagnosis usually clear.

But there is a longer or shorter period in all these diseases, usually in the earlier stages during which a condition of hepatic insufficiency (hypohepatism) and more rarely, hepatic hyperfunction (hyperhepatism) exists, which might be recognized by appropriate tests. The trend of modern investigation is in the direction of the development of practical functional tests of sufficient simplicity to enable the clinician to judge the functional capacity of the liver, before gross organic lesions or indubitable symptoms have appeared.

Tests for liver functional capacity may be profitably considered under five headings.[1] The first four are endocrinous, the fifth exocrinous. They are as follows: I. Disturbance of the glycogenic function. II. Disturbance of the ureagenetic function. III. Disturbance of the antitoxic function. IV. Disturbance of the hemapoietic function. V. Disturbance of the biliary function.

I. FUNCTIONAL TESTS TO DETERMINE DISTURBANCES OF THE GLYCOGENIC FUNCTION OF THE LIVER

Normally the liver cells retain in the form of glycogen almost all the glucose brought to them from the ali-

[1] See Les Procedes actuel d'etude de l'insuffisance hepatique. Gaz. d. hôp. Par. 1914, no. 25, p. 408 (Brule, Garban). To this article is appended an extensive bibliography.

mentary tract. Under certain conditions of liver insufficiency, glucose is not fixed by the cells, and passes immediately into the blood, producing a hyperglycemia, from whence it is excreted in the urine. This fact has been utilized as a basis for testing the glycogenetic integrity of the liver cell.

The name of Claude Bernard is closely linked with the history of the physiology of hepatic glycogenesis. Soon after the discovery by Claude Bernard of the rôle of the liver in carbohydrate metabolism, the use of sugars as tests for hepatic function began.

In applying the sugar test, different varieties of sugars have been employed, particularly saccharose (cane sugar), glucose, levulose, and galactose. The first sugar employed for this purpose was saccharose (cane sugar).

The sugar tests of hepatic function are four in number, as follows: 1. The Cane Sugar test. 2. The Glucose test. 3. The Levulose test. 4. The Galactose test.

1. The Cane Sugar Test. Colrat, Lepine Test

150 to 200 grams of cane sugar syrup are administered to the subject in the morning while fasting. The urine is collected every hour or two and examined for sugar with Fehling's solution or other means. The presence of glycosuria renders the test positive.

The cane sugar test was considered for a long time the best criterion of liver insufficiency. The clinical results of the test have, however, been contradictory. A rather weighty theoretical objection is the fact that cane sugar must be converted into glucose in the alimentary tract before it can be utilized by the organism, and the power of the intestinal juices to produce this

conversion is in each case an unknown quantity and therefore a source of error.

2. The Glucose Test

The patient takes in the morning on an empty stomach, 150 grams of pure dextrin-free glucose dissolved in 300 c. c. of water. The ingestion of this amount should not take over a quarter of an hour.

The urine is then collected every hour or two for ten hours in separate vessels and tested for sugar, the patient remaining on a milk diet during the time required by the test.

Castaigne has advised the following details with a view of perfecting the glucose test. For several days prior to the performance of the test, the subject should be kept on a certain known quantity of carbohydrate. The renal permeability should be investigated and the possibility of spontaneous glycosuria especially after meals eliminated.

The results of the glucose test have been rather conflicting and some investigators have appeared to find glycosuria following the test in apparently healthy subjects and its absence in certain cases of hepatic cirrhosis where the liver parenchyma would have been acknowledged on general clinical grounds to have been damaged.

3. The Levulose Test. Strauss Test

This test was introduced by Strauss [2] in 1901 as a substitute for the saccharose and glucose tests.

[2] Berl. klin. Wchnschr., 1898, XXXV, p. 398, and 1899, XXXVI, p. 159; Deutsch. med. Wchnsch., 1901, XXVII, p. 756, also 1903, XXXIX, p. 1780.

To apply the test 100 grams of levulose are given in the morning on an empty stomach and the urine evacuated every four hours thereafter for a day and examined for sugar by the fermentation test or polariscope.

Owing to the high price of levulose, honey, which contains a large percentage of it, has been advised as a substitute.

A normal person should tolerate 100 grams of levulose without levulosuria.

The rationale of this test was founded on the experimental work of Sachs,[3] who found that frogs whose livers had been removed had a lower tolerance for levulose than intact controls. With dextrose and galactose this was not the case. It was contended therefore that there is no mechanism besides the liver capable of handling levulose, while there is such an extra hepatic mechanism in the case of glucose.

Immediately after its introduction, this test came into pretty general use and was commended by Ferranini [4] v. Halasz,[5] Bruining,[6] and others, and was condemned by Landsberg,[7] Churchman,[8] and others. Much was expected from the levulose test because as above stated it was believed that the liver alone is concerned in levulose metabolism. However this may be, experience has apparently failed to substantiate the hopes which the test originally inspired and it is not now believed that the levulose test is essentially superior to other sugar tests of hepatic function.

[3] Zeitschr. f. klin. Med., 1899, XXXVIII., p. 87.
[4] Zeitschr. f. inn. Med., 1902, XXIII, p. 921.
[5] Wien. klin. Wchnschr., 1908, XXI, p. 44.
[6] Berl. klin. Wchnschr., 1902, XXXIX, p. 587.
[7] Deutsch. med. Wchnschr., 1903, XXIX, p. 563.
[8] Johns Hopk. Hosp. Bull., 1912, XXIII, p. 10.

4. The Galactose Test. Bauer's Test

Forty grams of milk sugar dissolved in 400-500 c.c. of tea are taken in the morning on an empty stomach. The urine is passed every four or five hours thereafter and examined for sugar.

In icterus gravis and catarrhal jaundice this test has been reported as giving fairly constant results.

Bauer [9] considered the galactose test especially adapted to determining the condition of liver function in catarrhal jaundice. The amount of sugar recovered in the urine after the galactose test was found to be greater in catarrhal jaundice than in obstructive jaundice, consequently it was supposed that the test would be of importance in differential diagnosis between the two conditions. This opinion was upheld and confirmed by Bondi and König,[10] Riess and Jehn,[11] and Hirose.[12]

Outside of catarrhal jaundice the results were pronounced inconstant by Falk and Saxl,[13] v. Frey,[14] and others.

Conclusions concerning the Sugar Tests.—It may be said that the investigation of hepatic insufficiency by any or all of the sugar tests, is to be regarded merely as supplementary or complementary to other means of investigation, since the results of these tests alone are not conclusive. Nevertheless the results which may be obtained by their help when associated or corelated with

[9] Wien. med. Wchnschr., 1906, LVI, p. 2557.
[10] Wien. med. Wchnschr., 1910, LX, p. 2617.
[11] Deutsch. med. Wchnschr., 1912, XXXVIII.
[12] Deutsch. Arch. f. klin. Med., 1912, CVIII, p. 187.
[13] Ztsch. f. klin. Med., 1911, LXXIII, p. 131, 325.
[14] Ztschr. f. klin. Med., 1911, LXXII, p. 383.

those obtained by other methods are of sufficient value to justify their retention in clinical medicine.

It is now understood that the mechanism whereby alimentary glycosuria is produced is more complex than was formerly supposed, and that the liver is not the only organ involved in the process. Other tissues are now known to be concerned in glycofixation and mobilization. Furthermore, the individual coefficient of sugar utilization has been found to vary within quite wide limits, 50-350 grams of levulose for example. The coefficient varies also in the same individual for the different sugars so that in reporting results of sugar tests it is deemed expedient to specify the particular kind of sugar used.

The unknown factors of intestinal absorption and renal permeability complicate all sugar tests.

The sugar tests have been found positive, especially in severe bivenous cirrhosis, in icterus gravis and in cholelithiasis. They are, however, of no prognostic value.

A recent comprehensive study of the applicability of carbohydrates as tests for hepatic functional activity has been made by Bloomfield and Horwitz.[15] These authors call attention very properly to the factors which tend to render the sugar tests for hepatic function unreliable. The great theoretical stumbling block in the way of accepting the finding of the carbohydrate tests is the fact that extra-hepatic factors of considerable importance are concerned in the sugar regulating metabolism. Some of the glands of internal secretion take part in this. Certain lesions of the hypophysis may cause glycogenolysis and glycosuria, while with other hypophyseal lesions the sugar tolerance is in-

[15] Johns Hopk. Hosp. Bull., 1913, XXIV, p. 375:—a good bibliography is appended to this article.

creased.

The internal secretion of the pancreas is generally considered to exert an inhibitory effect on the mobilization of glycogen by the liver. The suprarenals have an accelerating effect on the same mechanism. The thyroid gland also takes part in sugar metabolism, the exact nature of which is unknown. Both the autonomic and sympathetic nerves likewise affect the mobilization of glycogen. These last facts, of course, tend to render the sugar tests less definite and satisfactory.

From a practical standpoint it cannot be denied that the sugar tests have certain disadvantages. It is difficult for patients to ingest the large quantities of sugars required without the occurrence in certain cases of nausea, vomiting and diarrhœa. Faulty absorption, intestinal fermentation, portal obstruction with collateral circulation, sugar retention due to nephritis, inconstancies in the diet also combine to render the results inaccurate.

It will remain for the future, however, to determine whether the sugar tests of hepatic insufficiency can be developed or modified in such a way that the rather numerous objections with which impartial observers are agreed the tests are encumbered, may be eliminated. In such an event these tests, which from an historical standpoint are so interesting, may take a definite place, even though subsidiary, in that important group of tests by which one seeks to obtain an insight into the functional integrity of the liver.

II. FUNCTIONAL TESTS TO DETERMINE DISTURBANCES OF THE UREAGENETIC FUNCTION OF THE LIVER

All the tests for hepatic function which deal with the ureagenetic activity of the liver are concerned with

the question of nitrogen metabolism. The liver plays a large and important part in this metabolism; perhaps not so exclusive a rôle, however, as was formerly supposed.

The ureagenetic function tests are, for the most part, merely studies of nitrogen metabolism. They consist mostly of estimations of the amount of nitrogen eliminated in the urine with accurate partition of this nitrogen into different groups, particularly urea and ammonia. The relation between the amount of nitrogen eliminated in these two forms when compared with the total nitrogen eliminated will afford valuable criteria for estimating the ureagenetic functional capacity of the liver.

The practical estimation of ureagenetic functional capacity of the liver involves much more complex series of chemical processes than were found to be involved in the investigation of the carbohydrate function by means of the sugar tests. Inasmuch as the first criterion of normal liver function from the standpoint of nitrogen metabolism involves the relation between the amount of urea excreted in the urine and the amount of total nitrogen excreted therein, the investigator must make two separate chemical analyses. First the exact amount of urea excreted must be estimated, and second, the exact amount of total nitrogen eliminated. In discussing these analyses, no attempt will be made to give any data beyond the description of such methods which, on account of their comparative simplicity and established accuracy, have become firmly established in the clinic. The estimation of total nitrogen in the urine by the standard method—that of Kjeldahl—requires a good deal of time and some technical skill. But it is a method which can be easily performed in any well-equipped laboratory attached to a hospital, and should be car-

ried out by a competent person. A rapid and accurate method of quantitative estimation of urea has been lately devised—that of Marshall—and this method has already become the standard one in the clinic.

The estimation of urinary ammonia, or ammonia nitrogen, is another step required in the study of the efficiency of the liver from the standpoint of nitrogen metabolism. Here also it is best for the clinician to familiarize himself with one method, preferably that of Folin, the details of which are further on discussed.

The actual administration of ammonium-bearing or amino-acid-containing substances to the patient, with the subsequent examination of the urine to determine the capacity of the liver to convert these substances into urea, is another method of clinical investigation of the liver function. Finally it is conceded that the estimation of rest or residual nitrogen in the blood serum may be utilized to determine whether or not the liver cell is capable of producing an adequate urea synthesis of nitrogen products in the body.

To recapitulate, we may tabulate the different methods of testing the ureagenetic functional power of the liver under the following heads and in this order they will be discussed: 1. Urea Elimination and Nitrogen Coefficient as Criteria of Ureagenetic Liver Function; 2. Augmentation of Urinary Ammonia as an Index of Ureagenetic Liver Function; 3. Amino-aciduria as a Criterion of Ureagenetic Liver Function; 4. Estimation of Residual Nitrogen in the Blood Serum as an Index of Ureagenetic Liver Function.

1. Urea Elimination and Nitrogen Coefficient as Criteria of Liver Function

Urea Elimination.—The liver has long been regarded as the chief source of urea, which substance is supposed

to be formed from ammonium salts, amino acids and products of nitrogenous catabolism.

The urea synthesis is supposed to be the work of the liver cell governed by ferments which it secretes.

It has long been recognized that in certain diseases of the liver the percentage of urea eliminated in the urine is lowered. Therefore diminution of urea excretion may sometimes indicate hepatic insufficiency and an estimation of the quantity of urea eliminated is therefore an available factor in testing the ureagenetic function of the liver cell.

The quantity of urea eliminated in the urine by a healthy adult in 24 hours is 25 to 30 grams. In cases of hepatic insufficiency this quantity may fall to 10, 5, 3 or .5 grams or even 0 in icterus gravis.

But before one can attribute a diminution of urea elimination to functional disorder of the liver, certain other factors of great importance must be taken into consideration. One of these is the functional capacity of the kidney. It is well understood that urea retention in the blood may be due to defect of kidney permeability.

Another important factor is the amount of proteid intake. Before attempting to draw any conclusions with respect to the relation of urea elimination to the ureagenetic functional capacity of the liver it will always be necessary that the individual to be tested shall be placed for a sufficient length of time upon a fixed ration in which the amount of proteid is known and invariable.

The Nitrogen Coefficient.—The relation of urea nitrogen to the total nitrogen excreted in the urine is known as the coefficient of nitrogen elimination. This coefficient may be important since under certain circumstances its diminution may constitute a valuable sign of ureagenetic hepatic insufficiency.

The nitrogen coefficient is usually expressed by the following fraction:

$$\frac{\text{N. urea}}{\text{N. total}}$$

the arithmetical relation in other words between the amount of nitrogen eliminated in the urine as urea and the total quantity of nitrogen eliminated. This coefficient will diminish in proportion to the diminution of urea nitrogen.

According to some authors a diminution of the nitrogen coefficient is absolutely constant in hepatic insufficiency.

The normal figures of the nitrogen coefficient vary from 85% to 95%. In icterus gravis it has been found reduced to 40; likewise in phosphorus poisoning.

But just as in estimating the percentage of urea in the urine and considering the same as an index of hepatic function, so also in determining the coefficient of nitrogen elimination for the same purpose the patient must be placed on a fixed and invariable proteid regimen, though the absolute quantity of proteid taken is negligible.

It must also be previously known that there is no deficiency in renal permeability. To determine the coefficient of nitrogen elimination, two operations must be performed. First, a quantitative estimation of urea must be made, from which the calculation of urea nitrogen may readily be accomplished. Secondly, the total nitrogen eliminated in the urine must be estimated and this part of the operation is, unfortunately, somewhat difficult and time-consuming. The simplest known means for performing these operations will be given.

Quantitative Estimation of Urea in Urine.—Several

methods are in use for determining quantitatively the amount of urea in urine. One of the most frequently used is the hypobromite method, using the ureometer of Doremus. In this method nitrogen is set free by sodium hypobromite and measured in the apparatus. The results obtained by this method are extremely inaccurate and it is not used, therefore, where absolute results are required.

Three other methods which are much more dependable have been in use for some years in the laboratories. These are: 1. The Morner-Sjoqvist;[16] 2. Folin's method;[17] and 3. Schöndorff's method.[18] The details of these methods may be found in any modern text book on laboratory methods. We shall not give a description of them here.

Marshall's Method of Urea Estimation.—Quite recently a rapid chemical method for the estimation of urea in urine has been introduced by Marshall of Johns Hopkins.[19] It depends upon the conversion of urea into ammonium carbonate by means of an enzyme prepared from soy bean. This enzyme is called urease because of its facility in effecting this conversion. Urease is found in some bacteria and fungi. The following formula represents the chemical decomposition produced:

$$CO\begin{matrix} NH_2 \\ NH_2 \end{matrix} + 2H_2O = CO\begin{matrix} ONH_4 \\ ONH_4 \end{matrix}$$

The presence of urease in soy bean (glycine hispida) was first observed by Takeuchi in Japan. Its applica-

[16] Skand. Arch. f. Phys., 1891, II, p. 438; Zeit. f. phys. Chem., XVII, p. 140.
[17] Zeit. f. phys. Chem., 1901, XXXII, p. 504.
[18] Arch. f. d. Ges. Phys., 1896, LXII, p. 1.
[19] Jour. of Biol. Chem., 1913, XIV, no. 3; 1913, XV, no. 3.

tion to the quantitative estimation of urea is due as above stated to Marshall.

A convenient form of the enzyme urease is now to be found upon the market under the name of urease (Dunning). It is supplied in convenient 25 milligram tablets put up 40 tablets per package by Hynson Westcott & Co., pharmaceutical chemists of Baltimore, Maryland.

Urease (Dunning) is a fine, almost white, powder, with little taste or odor, soluble in slightly alkaline water.

The apparatus and material required for the estimation are as follows: Four 200 c.c. Erlenmeyer flasks with cork stoppers; one 50 c.c. glass-stoppered burette; one 5 c.c. bulb pipette; one small glass mortar; 100 c.c. of solution of methyl orange; 1000 c.c. of decinormal solution of HCl; 50 c.c. toluol and a package of urease (Dunning) tablets.

Put 1 or 2 c.c. of toluol into each of two Erlenmeyer flasks of 200 c.c. capacity. Into one of the flasks introduce exactly 5 c.c. of a specimen of urine and 100 c.c. of distilled water; stopper flask with cork. Crush a urease tablet in a small glass mortar and dissolve in about 5 c.c. of water. Transfer this solution without loss into the other flask containing toluol and rinse mortar with several portions of distilled water until about 100 c.c. have been added to the contents of the second flask. Add 5 c.c. of the urine and stopper with a cork.

Each flask is now thoroughly shaken and allowed to stand at room temperature over night or at least 8 hours. If it is necessary to get more rapid estimations two tablets are used instead of one and the mixture digested at 40°C for an hour.

The test may indeed be completed in 15 minutes by

using only 1 c.c. of urine, two tablets and digesting at 40° Centigrade for 15 minutes. The factor would in this case be 3 instead of .6, as will be seen later by carrying out the longer time limit.

After the lapse of the time set the two solutions are titrated to a distinct pink color with decinormal HCl, using methyl orange as indicator.

The urease has converted the urea present in the urine into ammonium carbonate. The amount of ammonium carbonate formed by the urease is indicated by the quantity of standard HCl solution required to exactly neutralize the contents of the flask containing urease minus the quantity required for control specimen.

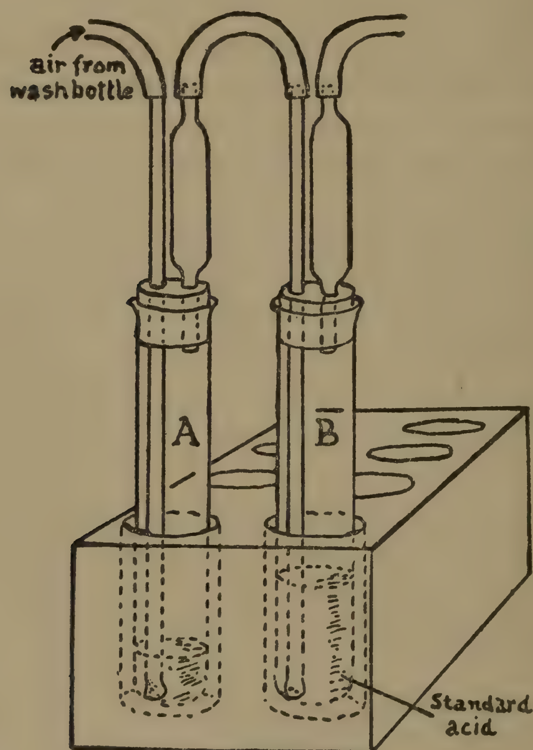
According to the chemical formula representing the conversion of urea into ammonium carbonate (*v. s.*) it may be seen that 60 grams of urea are converted (by urease) into 96 grams of ammonium carbonate. This amount (96 grams) of ammonium carbonate would require 72 grams of standard HCl solution to neutralize it.

As this quantity of HCl solution (72 grams) is contained in 20,000 c.c. of $^{n}/_{10}$ HCl solution and is equivalent to 60 grams of urea represented by 96 grams of ammonium carbonate, then 1/20000 of the quantity of 1 c.c. of $^{n}/_{10}$ HCl solution will be equivalent of 1/20000 of 60 grams, equal .003 ($60 \div 20000 = .003$).

Therefore each c.c. of decinormal HCl solution required to neutralize the enzyme treated specimen minus the number of c.c. required to neutralize the control specimen represents .003 of urea, and as the 5 c.c. specimen is the $^{1}/_{200}$ part of a liter, multiply the number of c.c. of $^{n}/_{10}$ HCl solution in excess of the control requirements by the factor .6 ($.003 \times 200 = .6$) to find the urea per liter when estimating the daily output.

One part of nitrogen is equivalent to 2.143 parts of urea.

Determination of Urea in Urine—Van Slyke's Method.—1. One-half c.c. of urine is measured into tube A (see illustration). Add 1 c.c. of a 10 per cent. solu-



Apparatus for determining the urea content by means of urease.

tion of *Urease* (or 1 tablet, which will quickly disintegrate and dissolve), and then 5 c.c. of water.

2. Add 2 drops of caprylic alcohol, to prevent

foaming. Close with the stopper. Allow to digest 15 minutes at 20° C., or for a shorter time if the temperature is between 20° C. and 50° C.

3. Measure 25 c.c. of N/50 hydrochloric or sulphuric acid into tube B, add 2 drops of caprylic alcohol, and then 1 drop sodium alizarinsulfonate as indicator. Connect the tubes as shown.

4. When digestion is complete, an air current is passed for a half minute to sweep over into B any small amount of ammonia which, during digestion, has escaped into the air-space of tube A.

5. Open A and add 4 to 5 gm. of potassium carbonate.

6. Pass an air current rapidly through the tubes until all ammonia has been carried over into the acid in B. (The time required depends on the rate of aeration, which varies from five minutes to a half hour.)

7. When aeration is complete, the excess of acid in B is titrated with N/50 sodium hydroxide.

8. The difference in c.c. of N/50 acid represents the urea plus ammonia nitrogen of the urine sample.

9. To determine the ammonia nitrogen alone, measure 5 c.c. of urine into A. Add potassium carbonate at once and aerate as before. Titrate the excess of acid as in paragraph 7.

Calculation of Result.—The number of c.c. of N/50 acid neutralized in operation 9, divided by ten, and subtracted from that neutralized in operation 7, represents the N/50 acid neutralized by the urea nitrogen alone in 0.5 c.c. of urine.

This result multiplied by 1.2 gives the number of grams of urea contained in 1,000 c.c. of urine.

For example:

N/50 acid neutralized by 0.5 c.c. urine (No. 7) .... = 9.50 c.c.
N/50 acid neutralized by 5.0 c.c. urine divided by 10 (No. 9)............ = .36 "
N/50 acid neutralized by 0.5 c.c. urine alone .... = 9.14 "
Result: 9.14 × 1.2 = 10.96 grams of urea per 1,000 c.c. of urine.

Determination of Urea in Blood, Spinal Fluid, Etc. —Van Slyke's Method.—"Urease is particularly valuable in permitting a simple and accurate determination of urea in the blood because its action is so specific that it attacks none of the other constituents."

The blood when drawn is mixed with 1 per cent. of dry potassium citrate (or the equivalent in citrate solution), to prevent clotting.

Three c.c. of the blood or fluid are mixed with 1 c.c. of the *Urease* Solution (or one soluble tablet added). Add 5 c.c. of water, then 5 drops of caprylic alcohol to prevent foaming. Allow to digest for ten minutes at 40° C. Measure 15 c.c. (mils) of N/100 acid into tube B. Proceed as for analysis of urine, except that the titration is made with N/100 NaOH.

Calculation of the Result.—Each c.c. of N/100 acid neutralized corresponds to 0.01 per cent. of urea.

Grams of urea per 100 c.c. (mils) of blood = 0.01 × c.c. of N/100 acid.

Urea nitrogen per 100 c.c. of blood = 0.00466 × c.c. of N/100 acid.

USEFUL REFERENCES TO LITERATURE ON UREASE

D. D. Van Slyke and G. E. Cullen, *Proc. Soc. Exp. Biol. and Med.*, 1913, Dec. 17; *Proc. Soc. Biol. Chem.*, 1913, Dec. 29; *Journ. Biol. Chem.*, 1914, Vol. XIX, pp. 141, 211; 1916, XXIV, p. 117.

D. D. Van Slyke and Gotthard Zacharias, *Journ. Biol. Chem.*, 1914, Vol. XIX, p. 181.

Estimation of Total Nitrogen in the Urine. Kjeldahl's Method.[20]—Ten c.c. of urine are carefully measured into a Jena glass, round-bottom flask. Add a few drops of concentrated solution of sulphate of copper, 15 c.c. of H_2SO_4 and 10 grams of potassium sulphate.

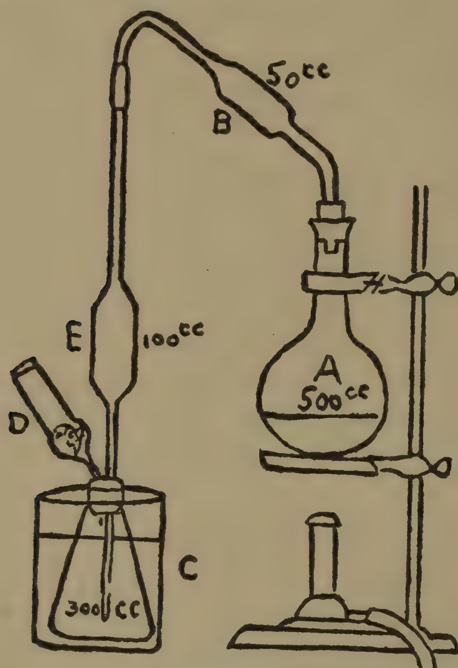
The flask is supported in an inclined position to prevent loss by spurting. The mouth of the flask is loosely closed by a glass bulb blown on end of a piece of glass tubing.

The flask may be conveniently supported by a thick piece of asbestos board with a hole in the center of a size to permit the flame to come in contact only with the portion of flask covered by fluid. Wire gauze protects the flask from direct contact with the flame.

The flask should now be gently heated over a Bunsen flame for half an hour. When foaming ceases the flame is raised until the acid begins to boil gently. The whole heating process is carried out under a hood because of sulphurous acid fumes which are given off. When the fluid in the flask is colorless or pale green, oxidation is complete. This requires about two hours. The acid and the oxidizer ($CuSO_4$) convert all nitrogenous matter into ammonium sulphate. The flask is then cooled. The next step is distillation. The same or a larger

[20] Zeit. f. Chem., 1883, XXII, p. 378.

Jena glass flask may be used, but preferably a copper apparatus which will not break. Two hundred c.c. of water are added and enough 30% NaOH solution to make the mixture strongly alkaline. Ammonia is set



KJELDAHL APPARATUS

free by the action of the alkali. This is distilled over in 80 c.c. of decinormal H_2SO_4, which has been accurately measured into a flask. The flask containing the original solution is heated until about 2/3 of it have passed over and there is considerable bumping from the separation of sodium sulphate. This usually requires about thirty minutes. Bumping may be diminished by adding fragments of pumice, granulated zinc or talcum powder at the beginning of distillation. A simple form of apparatus can be extemporized in the

laboratory. [See Illustration on page 34.]

The tube D contains a few glass beads and some of the H_2SO_4 is poured over these to prevent the escape of any ammonia. A few drops of methyl orange are added to the pearls and the flask C to indicate alkalinity, in which event more acid is to be added promptly. D is not necessary if a Liebig condenser is used.

The tube B prevents the alkaline fluid in A from spurting over into E. The tubes E and B are made of broken pipettes of 50 or 100 c.c. capacity.

The decinormal acid into which the ammonia has condensed is titrated with $^{n}/_{10}$ NaOH. Methyl orange is used as an indicator.

Subtract the number of c.c. of decinormal NaOH used from the number of c.c. of acid taken and the remainder will give the amount of ammonia distilled over, for every c.c. of acid neutralized by the ammonia is equivalent to so much decinormal ammonia.

Decinormal ammonia contains 1.4 grams of nitrogen to the liter or .0014 gram per one c.c. The amount of nitrogen can therefore be determined by multiplying the number of c.c. of acid neutralized by .0014. This gives the nitrogen in grams for 10 c.c. of urine used.

The description of the method given above is taken from Wood's Chemical Diagnosis, N. Y., 1909, p. 408.

2. *Augmentation of Urinary Ammonia as an Index of Ureagenetic Liver Function*

Ammonia, amino acids and carbonates constitute the last intermediaries in the metabolic processes by which the body proteids are catabolized into urea. As before stated, the liver has been regarded as the chief factor in this conversion.

If the functional capacity of the liver is deficient, the amount of nitrogen eliminated in the form of ammonia will be increased. Normally, .7 gram of ammonia are excreted by the urine in 24 hours. In hepatic insufficiency the amount may be doubled or trebled.

The relation of ammonia nitrogen to total nitrogen in the urine is normally 3 or 4 parts per hundred. Under pathological conditions, however, it may rise to 30 parts per 100 and such an increase may, under certain circumstances, indicate hepatic insufficiency. In states of acidosis the ammonia nitrogen is also increased.

It is often useful to make a comparison between the amount of nitrogen excreted as ammonia and the nitrogen eliminated as urea. This is done as follows: The molecular weight of ammonia NH_3 is 17, the nitrogen fraction of ammonia is, therefore, $^{14}/_{17}$.

The molecular weight of urea $CO\begin{matrix} NH_2 \\ \\ NH_2 \end{matrix}$ is 60.

The nitrogen fraction is $^{28}/_{60}$ or $^{7}/_{15}$.

The amount of ammonia in grams in a given sample is estimated by the formalin method (*v. s.*), and $^{14}/_{17}$ of this represents the ammonia nitrogen. The urea is calculated in grams in the same sample of urine, by Marshall's method, and $^{7}/_{15}$ of the weight is nitrogen. Under normal conditions the ammonia nitrogen is about $^{1}/_{20}$ of the urea nitrogen.

Estimation of Ammonia Nitrogen in the Urine. The Formalin Method.—The estimation of acidity is the first stage in the estimation of ammonia nitrogen. Proceed as follows:

Measure out 25 c.c. of urine into a beaker and dilute with about double the volume of distilled water. Add 2 or 3 drops of phenolphthalein. Run in $^{n}/_{10}$ NaOH from a burette until a faint permanent pink color is

produced. Note the number of c.c. of NaOH used. Measure about 10 c.c. of commercial (40%) formalin into a second beaker. Add phenolphthalein. Neutralize exactly with $^{n}/_{10}$ NaOH. Add the neutral formalin to the neutral urine. The pink color disappears. Run in $^{n}/_{10}$ NaOH until the pink color returns. Note the number of c.c. of NaOH used. The result is calculated in terms of $^{n}/_{10}$ NaOH for the acidity. That is to say the acidity of the urine is given as the number of c.c. of $^{n}/_{10}$ NaOH required to neutralize 100 c.c. of urine to phenolphthalein. Thus if 10 c.c. of soda were used in the first titration to neutralize 25 c.c. of urine, the acidity of the urine is $^{10}/_{25} \times 100 = 40$. The ammonia result should be expressed in grams of ammonia per 24 hours. The number of c.c. of soda used in the second titration of the urine is the equivalent of the number of c.c. of ammonia present in the 25 c.c. of urine. Supposing the number of c.c. of soda used in the second titration to have been 10, then 10 c.c. $^{n}/_{10}$ NaOH $=$ 10 c.c. $^{n}/_{10}$ $NH_3 = 10 \times .0017$ gm. NH_3. Therefore the

$$\text{ammonia passed in the 24 hours} = \frac{10 \times .0017 \times 24 \text{ hours urine in c.c.}}{25}$$

The reaction depends upon the combination of the ammonium salts with formaldehyde to form urotropine and the consequent liberation of the acids previously combined with ammonia.

Experimental Provocative Ammoniuria.—This test is based upon the fact that normally, the liver transforms all ammonia transported to it into urea. If there is pathological alteration of the liver cell, this ammonia will not be transformed and the quantity of ammonia in the urine increases.

Before applying the test of provocative ammoniuria

in a given case the total ammonia excretion in 24 hours should be estimated over two days. Meanwhile the patient is put upon a fixed regimen.

In the morning, after having urinated, the subject is given 6 grams of ammonium acetate. The urine for the next 24 hours is collected and the ammonium content estimated (*v. s.*) which can be compared with that found prior to the test.

The value of this test is variously estimated. Some have concluded that any considerable increase of ammonia in the urine after the ingestion will always indicate an impairment of the functional integrity of the liver cell. It is only claimed to be of value when positive. Others, on the contrary, have insisted that in spite of very advanced disease of the liver the ammonium salts ingested continue to be transformed into urea.

3. *Aminoaciduria as a Criterion of Ureagenetic Function*

The existence of considerable quantities of amino acids, leucine and tyrosine, in the urine in liver diseases was noted in 1866 by Frerichs. In 1907 Glaessner [21] showed that in disease of the liver there is usually an increase in the relation of amino nitrogen to total nitrogen in the urine. Normally this relation, or so called coefficient, of aminoaciduria has been shown to vary from .5 to 3.5 per 100. In diseases of the liver (cholelithiasis, cirrhosis), the ratio may rise to 11 or even 13 per 100.

Labbe and Bith have estimated the amount of amino acids in the blood serum and have found it increased in certain liver diseases.

Hyperaminoaciduria, according to Brille and Gar-

[21] Zeit. f. Exper. Path. Therap., 1907, IV, p. 336.

ban,[22] is not necessarily an indication of liver disease. It may occur during states of rapid emaciation, cachexia, pneumonia, typhoid fever and diabetes complicated with acidosis.

These authors agree, however, that in all these states it may not be improbable that the appearance of aminoaciduria is dependent upon a disturbance of the function of the liver cell.

Provocative Aminoaciduria.—The principal substances which have been used are glycocol, alanin, asparaginic acid, and commercial peptone. These amino acids, also peptone, have been employed by different experimenters.[23] The patient is given certain quantities of these substances and the amount of amino-acid excreted in the urine carefully measured.

If the proper care has been taken in the days which precede the test to establish the normal amino nitrogen coefficient for the individual and to keep the subject under observation upon a fixed and invariable diet, then provocative aminoaciduria tests may be of value. They will frequently show a marked increase of the coefficient after the ingestion of the amino-acids (or peptones) as compared with the coefficient prior to the test, and the marked increase according to some authors will only occur when the liver parenchyma is diseased.

The insuperable difficulty in these methods of estimating liver function arises from the fact that the estimation of amino nitrogen in urine and blood requires very complicated chemical manipulations which effectually prevent their introduction into clinical medicine.

[22] Gaz. d. Hôp., 1914, LXXXVII, p. 405.

[23] Ztschr. f. klin. Med., 1910, LXXI, p. 261; 1911, LXXIII, p. 325.

4. Estimation of Residual Nitrogen in Blood Serum as an Index of Hepatic Function. Chauffard Brodin Test [24]

The quantity of residual nitrogen in blood serum is obtained by subtracting the urea nitrogen from the total nitrogen estimated in dealbuminized serum. Residual nitrogen is made up of ammonia, amino-acid, uric acid, etc. It has been contended that the elaboration of residual nitrogen products is exclusively dependent upon the liver. In normal persons the residual nitrogen is always below 10 grams per liter of serum. In many acute and chronic diseases of the liver, the residual nitrogen has been found above 10 grams, the amount being in proportion to the gravity of the hepatic lesion. The diet and condition of the kidney are negligible.

The estimation of the amount of residual or rest nitrogen in the blood serum has come to be regarded as of more importance as a test of kidney than of liver function. For this reason a more complete account of the method and its interpretation will be found later under that head.

General Summary of the Value of Ureagenetic Function Tests.—In the first place it must be admitted that some of these ureagenetic tests are complicated to such an extent that they cannot be carried out without the assistance of an expert chemist, thus diminishing their applicability to clinical work.

Further than this it must likewise be admitted that many physiological causes of error exist which militate against a too strict interpretation of results. Other factors besides the liver collaborate in nitrogen metab-

[24] Jour. Biol. Chem., 1912, XII, p. 301; Jour. Amer. Chem. Soc., 1913, XXV, p. 1567.

olism.

Notwithstanding these valid objections it must be admitted that the study of nitrogen has given some useful results and it is to be hoped that future investigations may bring a greater degree of order from the now more or less confused and contradictory material which comprises our stock of knowledge today concerning the true relation of the liver to nitrogenous metabolism.

When this day arrives the clinician will be better able to interpret the results obtained from ureagenetic tests than he is at the present time.

III. FUNCTIONAL TESTS TO DETERMINE DISTURBANCE OF THE ANTITOXIC FUNCTION OF THE LIVER

Poisons which obtain access to the organism through the portal circulation are fixed and destroyed under normal circumstances by the liver cells. It is natural, therefore, that this important function should be employed as a basis for testing the integrity of the organ.

The first efforts made in this direction consisted in estimating the toxicity of urine and blood serum. An increase of toxicity of these bodies, it was held, indicates diminished toxicopexic power of the liver parenchyma. Tests of this character are carried out upon lower animals. The urine to be tested being injected intravenously into rabbits according to Bouchard's [25] method, and blood serum or urine into the cerebrum of rabbits according to Widal's method. The numerous sources of error and the technical complications surrounding these two methods have prevented their introduction into clinical medicine and they need not be

[25] See also Estimation of Urinary Toxicity as a Test of Renal Function.

described.

The methods which are in use to determine the antitoxic functional power of the liver are two in number. They are as follows: 1. The Methylene Blue Test of Chauffard and Castaigne. 2. Estimation of Indicanuria, Spontaneous and Provocative.

1. Methylene Blue Test of Toxopexic Function of the Liver. Chauffard, Castaigne Test [26]

When the liver cell is unable to properly arrest and fix poisons, it reacts to the passage of methylene blue through its parenchyma by an intermittent elimination.

Test.—Inject 1 c.c. of 5% solution of methylene blue subcutaneously. Collect and examine the urine in half an hour, then every hour. Normally at the end of half an hour after injection the urine becomes colored, the color rising to maximum in 3 or 4 hours, disappearing in about 50 hours. If the elimination when it commences instead of being continuous occurs in cycles, that is intermittently, the test is positive and may indicate hepatic insufficiency.

It must be acknowledged that the value of this test is greatly diminished by the fact that the state of renal permeability must be considered, since this will have a predominant influence upon the quantity and rapidity of elimination. Where this factor is known the test may be of value.

Roche's Modification of Chauffard's Methylene Blue Test.—In this test the methylene blue is taken internally instead of being given hypodermically. .002 gm.

[26] Presse Méd., 1898, April 23; Jour. de Physiol. Path., 1899, May. See also Babaliantz, Thèse de Genève, 1912.

of methylene blue is swallowed at eight o'clock in the morning on an empty stomach. The substance is given in capsule. The urine is collected every four hours in separate vessels. If the urine of the second recipient is clearly colored it will denote inability of the liver to retain the pigment and consequent hepatic insufficiency. Normally this amount of methylene blue should be completely arrested and fixed by the liver so that no coloring matter appears in the urine after its administration. If the test is positive, the urine, especially that passed four to eight hours after administration, will be colored green.

Unfortunately the condition of the kidney must also be reckoned with in applying this test, as failure of elimination of the dye may be due to renal impermeability.

The same remarks with respect to renal permeability apply in interpreting the Roche modification as when the dye is given by hypodermic injection. It can never be known a priori whether a failure of elimination is due to renal insufficiency or to normal fixation of the dye by the liver cell. In other words, the state of the kidney function must be previously known or ascertained.

2. Indicanuria Spontaneous and Provocative as a Means of Testing the Integrity of Hepatic Fixation

Spontaneous Indicanuria.—It has been urged that the normal liver is always capable of arresting and destroying indican which is formed in the intestine as a result of putrefaction of albuminoids. Therefore the spontaneous presence of indican in the urine has been held to be evidence of hepatic insufficiency.

Provocative Indicanuria.—.001 gm. of indol is ingested in the morning on an empty stomach. The urine is collected every four hours and examined for indican. The individual to be tested should be put on a milk diet for a few days before the substance is ingested.

Tests for Indican in the Urine.—Qualitative.—The principle involved is to decompose the sodium or potassium compound of indoxyl sulphuric acid present in the urine by strong HCl and oxidizing this compound.

Obermayer's reagent is usually employed. This consists of strong HCl, sp. gr. 1.19, to which is added two parts per thousand of ferric chloride. The liquid is fuming yellow and keeps indefinitely.

Precipitate the specimen of urine to be tested with a small amount of lead acetate or subacetate, avoiding excess, and filter. This removes pigments. 15 c.c. of filtered urine are mixed with equal quantity of Obermayer's reagent and 2 c.c. of chloroform added. Cork or cap the tube with rubber, and slowly invert. The chloroform takes on a blue color whose depth will roughly show the amount of indican present.

Quantitative.—Strauss'[27] method is a good one. Twenty c.c. of urine are mixed with 5 c.c. of 20% lead acetate solution and filtered. Ten c.c. of filtrate (corresponding to 8 c.c. urine) are placed in a small graduated separatory funnel and mixed with 10 c.c. of Obermayer's reagent (*v. s.*).

Five c.c. of chloroform are added, the tube corked and gently shaken. This is repeated in two minutes. Pour the chloroform from the tube. Add 5 c.c. of chloroform and repeat the extraction. Continue until added chloroform remains colorless.

[27] Deutsch. med. Wchnsch., 1902, p. 299.

Two c.c. of united chloroform extracts are put in a small test tube of same diameter as tube containing standard solution. Chloroform is added drop by drop until colors are matched, against white background.

The standard solution is made by dissolving .001 gm. C.P. indigotin (Kahlbaum) in 1000 c.c. of chloroform. Portion is sealed in test tube and kept in the dark.

If the total amount of chloroform used for extraction is equal to a and the amount of chloroform used to dilute the 2 c.c. to the color of the standard tube equals x, the total amount of chloroform necessary to dilute all the chloroform used in extraction equals

$$a \times \frac{x}{2}$$

The total number of c.c. used in the extraction and in the dilution of the extraction mixture represents, therefore, a bulk containing .001 gm. of indigo.

In normal urines 5-10 c.c. of chloroform are usually all that is required to extract the whole amount.

To obtain the results in milligrams it must be considered that the amount of indigo extracted was from 8 c.c. of urine.

IV. SULPHO-CONJUGATION, PROVOCATIVE AS A MEANS OF TESTING THE HEPATIC FUNCTION (FOSTER AND KAHN)

Assuming that the toxic aromatic radicles as indol, skatol, phenol, etc., are normally conjugated in the liver with sulphuric and glycuronic acids, with the formation of ethereal sulphates, and their elimination in the urine,* Foster and Kahn have proposed the following method for the estimation of hepatic function:

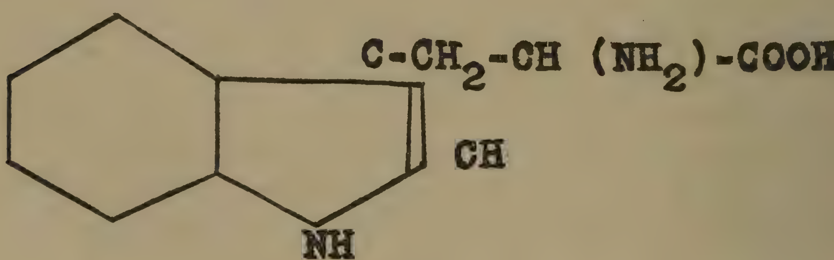
* Foster and Kahn, J. Lab. and Clin. M., St. Louis, 1916, 11, 1.

1. *The Sulpho-Conjugation*

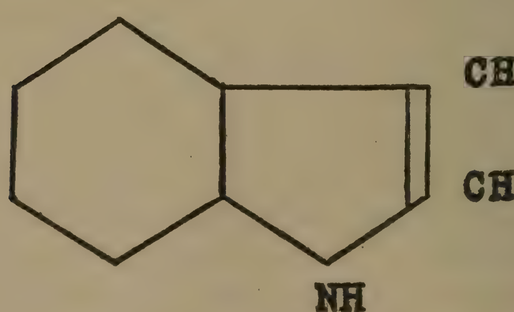
"This has helped us much in determination of liver function. We shall discuss this in detail.

"The toxic aromatic radicles produced by decomposition of protein are conjugated in the liver with sulphuric or glycuronic acid, and are then excreted in the urine. If we should take indol as an example, the following process would take place:

"Tryptophane, or beta-indol-alpha-amino-propionic acid is one of the products of decomposition and putrefaction of proteins. It is the mother substance of indol and skatol, etc. Upon breaking down of tryptophane, indol, which is very toxic, is produced.

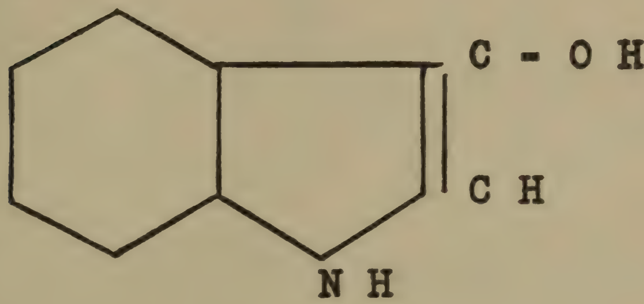


(Tryptophane)



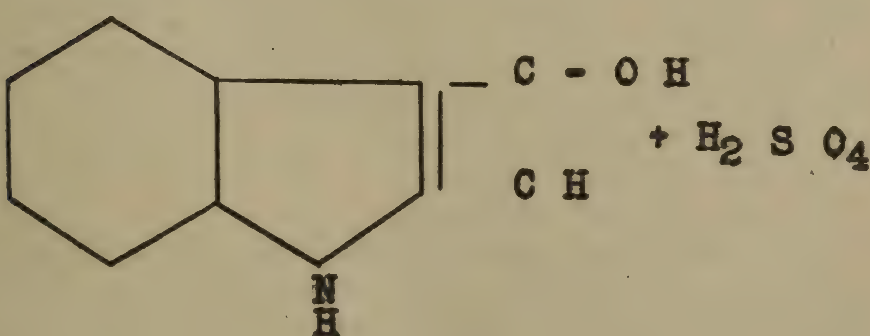
(Indole)

Indol is oxidized in the intestines to indoxyl.

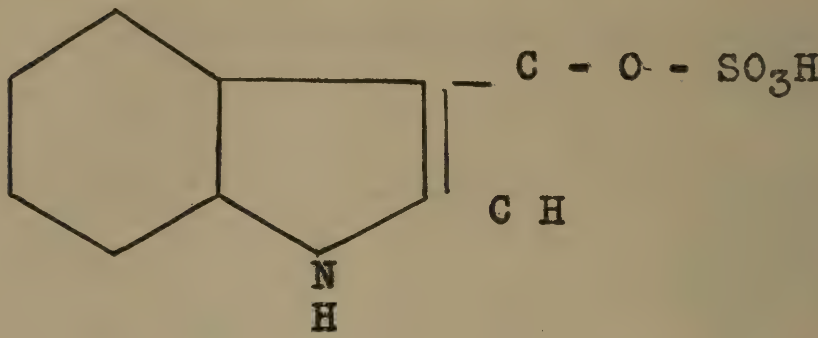


(Indoxyl)

If indol or indoxyl enters the general circulation marked toxinemia results with its concomitant symptoms. The protective mechanism of the body against this toxinemia is to conjugate the indoxyl with sulphuric acid in the liver, producing a substance which is almost nontoxic—indican.

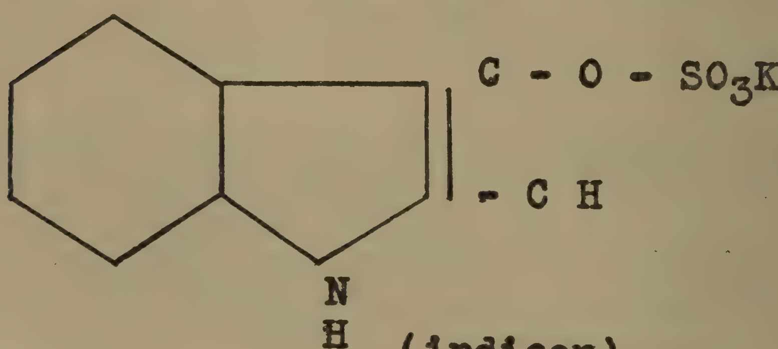


(indoxyl)



(indoxyl sulphuric acid)

In the presence of potassium salts:



(indican)

"Similar results are obtained with any of the aromatic radicles, as phenol, cresol, tyrosin, skatol, etc.

"It is well known that the total sulphur in the urine may be partitioned into three distinct fractions:

a. The Inorganic Sulphates.
b. The Ethereal Sulphates.
c. The Neutral Sulphur.

"It has been definitely established that, normally, the inorganic sulphates form about 70 per cent. of the total sulphur, and the remaining 30 per cent. are divided almost equally between the ethereal sulphates and the neutral sulphur.

The ethereal sulphates are the conjugated aromatic

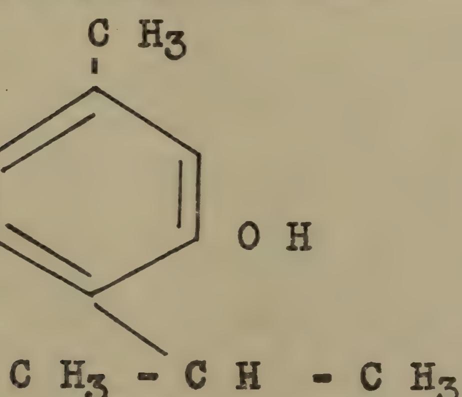
sulphonic acids. It is this fraction that is of special interest to us now.

It is, of course, impossible to rely upon the exertion of ethereal sulphates as a symptom of hepatic function. The proteins which are ingested daily give rise to their quota of aromatic radicals which influence the quantity of the conjugated sulphates. The condition of the intestinal flora plays a rôle in the formation of aromatic radicles, thus it is known that in intestinal putrefaction there is a marked increase in the conjugated sulphates excreted.

"We, therefore, adopted the following technic for the determination of liver function by means of the ethereal sulphate output:

"The patient received a dose of castor oil to clean out his bowels. He was then kept on a known diet for two days, during which time the urine was collected, preserved, and analyzed for total sulphur and ethereal sulphates.* On the third day the patient received a capsule containing one-half gram of thymol. The urine was collected for the next two days, preserved, and analyzed for total sulphur and ethereal sulphates.

"Thymol is iso-propyl-meta-cresol:



* The total sulphur was analyzed by Benedict's method; the Ethereal sulphates by Folin's method.

"If all the thymol were absorbed and if all the thymol were conjugated with sulphuric acid and none with glycuronic acid, the 0.5 gram of thymol would be excreted as 0.7666 gm. of thymol sulphuric acid. This would cause a marked increase in the percentage of ethereal sulphates. If the liver were not functionating properly, the thymol would not be conjugated, and the percentage of ethereal sulphates would be only slightly different from what it had been on the first two days.

"One objection to the study of the function of any organ as an index of disease of that organ is, that it is perhaps possible for the healthy part of the diseased organ to compensate and assume the work of the whole gland. In such a condition of course the functional output of the organ may be normal, and would be no index of the pathological anatomy of the organ. Under these circumstances only marked destructive changes would leave their impress on the functional activity of the organ.

ETHEREAL SULPHATE ELIMINATION BEFORE AND AFTER THYMOL ADMINISTRATION.

Case No.	Diagnosis	Total Sulphur gms.		Ethereal Sulphate Sulphur gms.		Ethereal Sulphate Sulphur % of Total Sulphur.	
		Before Thymol.	After Thymol.	Before Thymol.	After Thymol.	Before Thymol.	After Thymol.
1	Normal	2.0375	2.1295	0.2893	0.5646	14.2	26.8
2	Gastritis	1.9428	1.7427	0.1457	0.3380	7.5	19.4
3	Fracture	2.7467	2.5527	0.3131	0.6024	11.4	23.6
4	Congestion of liver	0.9852	1.0734	0.1753	0.7069	17.8	28.6
5	"	1.7345	1.6982	0.2480	0.3610	14.3	21.2
6	Gall-stones	2.7628	2.8075	0.7597	1.0303	27.5	36.7
7	" "	3.0042	2.6826	0.3965	0.8474	13.2	29.4
8	Cholecystitis	2.7807	2.6437	0.4866	0.7428	17.5	28.1
9	Atrophic cirrhosis	2.2328	2.3029	0.2791	0.3400	12.5	15.2
10	Tumor of liver	1.9492	1.8757	0.1637	0.3676	8.4	19.6
11	Cancer of liver	2.7526	2.6278	0.6083	0.6648	22.1	25.3
12	Syphilis of liver	2.8104	2.9075	0.3990	0.5437	14.2	18.7

"It has been our experience, however, that disturbances in the structure of the liver go hand in hand with disturbances of function, especially as is indicated by sulphuric acid conjugation of the aromatic radicles. We have found that in cirrhosis of the liver the conjugation of thymol with sulphuric acid does not take place to as marked an extent as in the normal state. This question is now being more fully investigated, and in the very near future we hope to make a more extensive report. Meanwhile, we have cited a few cases above.

"It will be observed that in the nonhepatic diseases, and in the non destructive diseases of the liver, a marked increase in the excretion of ethereal sulphates was observed on the day after the thymol administration. In diseases of the liver, like atrophic cirrhosis, cancer of the liver, or syphilis of liver, this organ has lost its power to conjugate the thymol with sulphuric acid. Case number 10 was a benign tumor of the liver, and it seems no destructive changes went on in the hepatic tissue. This case was in the service of Dr. E. B. Haworth.

"We hope to study this reaction more fully in experimental hepatisis, if possible."

V. FUNCTIONAL TESTS TO DETERMINE DISTURBANCES OF THE SANGUINOPOIETIC FUNCTION OF THE LIVER CELL

Some results of practical value have already come from tests of hepatic function based upon the hæmic activities of the liver. The whole question of the relation of the liver to the biology of the blood is very complex and not completely understood at the present time; but it is generally agreed that the liver is inti-

mately concerned in the elaboration of some of the constituents of the blood, particularly fibrinogen and certain of the ferments.

The hæmic tests so far proposed have dealt with two physiological aspects of the relation between the liver and blood: first coagulability and second the presence of miscellaneous ferments. If the liver is in reality the principal source for the elaboration of fibrinogen any notable diminution of this substance in the blood will indicate a diminution of the functional integrity of the liver cells. With diminished fibrinogen and perhaps also fibrin ferment, a delay or deficiency in the coagulability of the blood will ensue.

One test of liver function will therefore consist in estimating the coagulability of the blood.

With respect to the tests for ferments in the blood it may be said that the estimation of lipase is so far the most important. The liver under normal circumstances inhibits the formation of this ferment so that hepatic insufficiency results in an actual increase in the amount of lypolytic enzyme in the blood.

The other ferment tests of hepatic function are apparently too subtle and uncertain to be of any considerable practical value.

The functional tests which are in use at the present day to determine the sanguinopoietic capacity of the liver are as follows: 1. Estimation of blood coagulation time, and estimation of fibrinogen. 2. Estimation of fibrinolysis time. 3. Estimation of the amount of lipase in the blood.

1. Estimation of Blood Coagulation Time as an Index of Liver Function

Prolongation of coagulation time of the blood is said to be present when the liver is physiologically de-

fective and consequently the estimation of coagulation time has been proposed as a simple and effective means of determining hepatic insufficiency.

Unfortunately while the relation of normal hepatic function to normal coagulation of the blood is undoubtedly intimate and important, it is also true that so many other factors besides the liver enter into the physiology of blood coagulation as to materially lessen its importance as a basis for functional estimation.

Coagulation time of the blood has also been suggested by Tettinger and Bachrach as a means of testing renal function.

Wright's Method of Estimating Coagulation Time.—The necessary apparatus consists of a series of capillary tubes, elastic bands, a beaker, a jug of hot and a jug of cold water, a watch with a second hand and a thermometer. The capillary tubes are of the same caliber and are provided with a 5 c. mm. mark. The procedure is as follows: Clean the patient's thumb with ether. Wrap a piece of elastic tubing round the thumb from the base nearly to the tip. Puncture the tip of the thumb with a sterile surgical needle. Draw up blood to the mark on the pipette. It is not essential to obtain the exact quantity of blood, slight variations in the amount being of less importance than rapid manipulation. Note the exact time by the watch. Stretch a flat elastic band over the ends of the tube to prevent entrance of water. Stand the tube in the beaker filled with water at 37° C. Stir the water occasionally with the thermometer and keep the temperature constant by adding hot or cold water.

Prepare three or four more capillary tubes in the same way, numbering each tube and taking the time of each. At the end of three minutes take out the first tube and blow out the blood. Give the second tube

3½ minutes and if the blood is still fluid give the third tube four minutes and so on. The tube from which the blood fails to be expelled by blowing gives the coagulation time. The normal time for blood coagulation is 3½ minutes.

The Fibrinogen Test. Whipple, Horwitz.[28]—20 c.c. of oxalated blood plasma are heated to 59° C. for 20 minutes. Fibrinogen is precipitated. The precipitate is isolated by centrifugation, washed with water, alcohol and ether, dried at 120° C. and weighed. A rough estimate of the amount of fibrinogen in the blood is made by clotting a little plasma with calcium, testing the toughness of the clot with a glass rod. The quantitative or weighing method is, however, better.

Normally fibrinogen exists in the plasma in the proportion of .30 to .40 gm. per 100 c.c. In case of liver deterioration, alteration or injury the amount will be found diminished. In some cases of cirrhosis the fibrinogen content has been found very low (.05 gm. or less).

2. *Estimation of Fibrinolysis Time as an Index of Hepatic Function. Goodpasture Test*[29]

Very recently, Goodpasture has called attention to the interesting and important fact that in chronic, atrophic, hepatic cirrhosis the blood possesses the power of completely digesting the clot in a few hours at body temperature.

The clot in normal blood remains undigested for days and sometimes even for weeks. Goodpasture believes that the dissolution of the clot is due to an

[28] Jour. Exp. Med., 1911, XIII, p. 136; also Johns Hopk. Hosp. Bull., 1913, XXIV, p. 207, 343.
[29] Johns Hopk. Hosp. Bull., 1914, XXV, p. 330.

enzyme. The activity of this enzyme is destroyed by heat and inhibited by normal serum. The fibrinogen content of the blood in his reported cases (four in number) was below normal. He suggests premature digestion of clot *durante vivo* may account for the frequency of spontaneous hemorrhage in atrophic cirrhosis. The Goodpasture test will, in all likelihood, be found of great value in estimating hepatic insufficiency in chronic cirrhosis. It will be interesting to observe what the test for fibrinolysis will show in early or latent cases of cirrhosis as well as in hepatic conditions generally.

Technic of Goodpasture Test.—Blood is drawn from an arm vein by means of the usual technic. The coagulation time is estimated.* A portion of blood is drawn into 1% solution of sodium oxalate to prevent clotting of the specimen. This last is centrifuged and 20 c.c. of supernatant plasma is used to determine fibrinogen content if this is desired. Otherwise the entire oxalated plasma is used for tests of coagulation time and fibrinolysis.

In testing the oxalated plasma for coagulation time, use 1 c.c. of plasma + 1 gtt. $CaCl_2$ (1%).

The original clot from the drawn blood and specimens of clotted oxalate plasma are placed in the thermostat at 37°. They are examined every hour. If the test is positive, the blood clot liquefies and is dissolved in 3½ to 5 hours.

In negative (normal) cases there is no digestion of the clot for several days, even where no aseptic precautions are taken.

* For other methods than Wright's consult modern laboratory manuals.

3. Estimation of Blood Lipase as an Index of Liver Function. Whipple's Test [30]

There exists normally in the blood a lipolytic ferment lipase. The percentage of lipase in normal blood is remarkably uniform. In certain diseases of the liver this ferment has been found to increase in amount indicating that under normal circumstances the liver inhibits its formation. Any considerable increase, therefore, of lipase in the blood has been held to indicate hepatic insufficiency. Whipple, in collaboration with Mason and Peightal, found that after acute injury of the liver from chloroform there was always found an increase in the lipase of serum or plasma. Sometimes this rise amounted to 1–2 c.c. of $^1/_{10}$ normal acid. Inasmuch as the content of lipase may increase five to eight times the normal under certain conditions of hepatic disease it was naturally suggested as a test of hepatic insufficiency.

The value of the test is chiefly qualitative rather than quantitative. It has been found of especially positive value in suspected eclampsia, chloroform poisoning, yellow atrophy and cholangitis. In cirrhosis of the liver there may be a subnormal lipase.

Tests for Lipase in the Blood. Lowenhart's [31] *Method of Estimating Lipase Is Usually Employed.*—Blood serum is collected in four tubes and a little toluene, .3 c.c., is added to prevent microbic contamination. Each tube contains 1 c.c. plasma or serum diluted with 4 c.c. of distilled water.

Two of these tubes are used as controls. To the others a little ethyl butyrate (butyric ether), .26 c.c.,

[30] Johns Hopk. Hosp. Bull., 1913, XXIV, p. 207; ibid., 343; ibid., 357.
[31] Amer. Jour. Physiol., 1902, VI, p. 331.

is added. The four tubes are shaken, corked and put in the thermostat at 38° for 18 to 24 hours.

At the end of this time the two control tubes are examined for their normal alkalinity by means of decinormal acid solution. The other two tubes are titrated for free butyric acid by decinormal alkali solution.

The total lipolytic activity is measured by adding the two figures, since the butyric acid formed had to first neutralize the normal alkalinity of the serum.

The exact method is as follows: After incubation the tubes are cooled in ice water, 3 drops of azolitmin added and then titrated in pairs to a neutral reaction, using $^1/_{10}$ normal acid and alkali. The two control tubes usually show the blood alkalinity to be .1 c.c. $^1/_{10}$ normal acid and the butyrate tubes show the acid production to be .1 to .2 c.c. above the neutral point. This means that the total lipolytic activity is .2 to .3 c.c. $^1/_{10}$ normal solution, that the plasma lipase has split up the ethyl butyrate to this amount. Normal plasma lipase is then in terms of $^1/_{10}$ normal acid equal to .20 to .30 c.c.

Ghedini's Test.[32]—This consists in an estimation of the power of the blood serum to convert glycogen into glucose. A reducing ferment supposed to be formed by the liver cells effects the conversion.

The Test.—Blood serum to be examined is added to a solution of glycogen. Serum from a known normal person is similarly treated and used as a control.

To both tubes is added a little sodium hydroxide, then potassium sulphocyanide, and the solutions filtered and examined with the polariscope.

If there is hepatic insufficiency the rotatory power of the serum will be less than the normal control.

[32] Gazz. degli ospedali, Milan, Jan. 12.

The factors upon which this test are founded are rather subtle and imperfectly understood; indeed it is by no means accepted that the liver is the unique or even the most important source of the ferment which affects the conversion of glycogen into glucose.

4. Application of Abderhalden's[33] Method to Estimation of Sanguinopoietic Functions of the Liver

Recently it has been held that destruction of liver parenchyma by disease (autolysis) gives rise to the presence of an excess of proteolytic ferments in the blood serum and this fact has formed a basis for testing the functional capacity of the liver.

The technic of the method is too complicated to admit of its use in clinical practice and the results obtained are as yet too meagre to justify any conclusions as to its value.

V. FUNCTIONAL TESTS TO DETERMINE DISTURBANCE OF THE EXOCRINOUS OR BILIARY FUNCTION OF THE LIVER

Bile is partly a secretion and party an excretion, for it not only plays a rôle in certain digestive processes, notably the splitting or absorption of fats, but it also contains certain waste products which escape in the feces. It is important to remember, however, that certain constituents secreted and excreted in the bile are reabsorbed by the intestine and carried back by the blood stream to the liver.

Perfectly fresh hepatic bile, in contradistinction to bile in the gall bladder, contains one pigment only, namely, bilirubin, and from this pigment others are

[33] See Breitmann, Zentrbl. f. innere Med., XXIV, 1913, p. 857.

gradually formed by processes of oxidation. Fresh human bile from the hepatic duct is golden yellow in color but becomes olive brown to grass green after remaining in the gall bladder and cystic duct, because of the presence of biliverdin, which is an oxidation product of bilirubin.

It is now universally accepted that the bile pigment bilirubin originates from the disintegrated hemoglobin of the red blood corpuscles. When these break up their contained pigment is carried to and fixed by the liver, where it is converted into an iron-free pigment, bilirubin.

It appears that the power of transforming blood pigment into bilirubin is not exclusively the property of the liver cell, since in old blood extravasions anywhere in the body a substance, hæmatoidin, is found which is chemically identical with bilirubin. Under ordinary physiological conditions, however, the liver seems to be the only place in the body where bilirubin is formed. It is not known whether the actual disintegration of red corpuscles is confined to the liver, but certainly this organ has the unique power of fixing the hemoglobin set free by hæmolysis, retaining its iron and converting it into bilirubin.

Under normal conditions bilirubin does not appear in the feces, but in its place is found a reduction product which is known as urobilin (stercobilin). The reduction of bilirubin to urobilin takes place in the intestine from nascent hydrogen liberated by bacterial action. Part of this urobilin formed in the intestine escapes in the stools. Another portion is reabsorbed by the intestinal mucosa as a protochrome,—urobilinogen. Part of this substance is eliminated in the feces. The remainder passes back through the portal circulation to the liver, where it is changed back into bilirubin to be

again reexcreted by the bile when the cycle is repeated.

A very minute amount of the urobilinogen absorbed by the bowel escapes in the urine. Under normal conditions this quantity is very small.

Soon after urobilinogen is eliminated by the urine, it changes to urobilin so that the urobilin is not present in freshly passed urine but only appears from the breaking down of urobilinogen by light and air.

Urobilin was recognized as a constitutent of pathological urines long before urobilinogen, from which it is formed by oxidation, was discovered.

Urobilinuria and urobilinogenuria may therefore be regarded as practically identical and due to the same substance in different forms. The pathological causes of both are the same.

It has been known for a long time, however, that urobilin appears in the urine in a comparatively large number of pathological conditions, among which may be mentioned the infectious diseases, particularly malaria and pneumonia, cirrhosis of the liver, lead poisoning, decompensated heart disease, pernicious anæmia, pulmonary infarction and visceral hemorrhages. Likewise certain drugs and poisons are known to produce urobilinuria. In obstructive jaundice, it often appears in the urine before bilirubin and may, in fact, alternate with this substance. Urines which contain much urobilin present a dark yellow color which may be imparted to the foam on shaking. They are thus quite like ordinary icteric urines.

The hepatic conditions in which urobilin appears in the urine fall into two groups: 1. Mechanical interference with biliary flow in the ducts. 2. Insufficiency of the liver cell. In the first group, if the obstruction is absolute as in some cases of stone, no bile reaches

the intestine and no urobilin is formed, hence no urobilinogen appears in the blood or urine. If the obstruction remains complete the bile pigment bilirubin begins to be absorbed by the blood and excreted in the urine. If the occlusion of the ducts is only partial, some bile reaches the intestine, urobilin is formed, absorbed and may appear as urobilinogen in the urine. On removal of the obstruction the full flow of bile in the intestine is resumed. The functional activity of the liver is sufficient to eliminate the urobilinogen brought to it by the blood from the intestine and hence there is no call for its elimination by the kidney and it becomes reduced to a mere trace in the urine or completely disappears therefrom.

As a sign of liver insufficiency urobilinogenuria may be absolute or relative. Absolute when the liver parenchyma is totally unable to eliminate urobilinogen and allows it to get into the blood; relative when an excessive breaking down of red corpuscles anywhere in the body overwhelms the liver with pigment which the cells are unable completely to convert into bilirubin. In other words, if the liver is insufficient it will be unable to excrete increased amounts of urobilinogen whether derived from intestinal urobilin or from blood pigments. In either case there will be an increase or accumulation of urobilinogen in the blood and it will appear in measurable amounts in the urine.

Icterus with the appearance in the urine of normal bile pigment (bilirubin) may be regarded as a further step in such a pathological process. Here the defect of excretory function of the liver is greater because of actual inflammatory obstruction of the biliary tract. Jaundice, as is well known, is the result of the absorption into the blood of the bile pigment bilirubin. Jaundice, whether obstructive or toxemic, is always due to

some lesion of the biliary excretory passages by which the flow of bile into the intestine is diminished or prevented and is always accompanied by some inflammatory state of the biliary tubules. Icterus, when well marked, becomes clinically quite evident from the discoloration of tissue which results. The demonstration of bilirubin in the urine is then superfluous. In such an event, icterus becomes an element in the semiology of hepatic disease and has to be evaluated with other symptoms in making a diagnosis.

The presence of small quantities of bile pigment (bilirubin) in the urine is, however, an earlier sign of exocrinous hepatic insufficiency than icterus. For this reason the tests for bile pigment in the urine become tests to a certain extent at least of hepatic disease and perhaps insufficiency.

To summarize the above facts in their relation to the estimation of the external secretory function of the liver it may be said that disturbances of this function are identified by tests which disclose the presence in the urine of the three substances above mentioned, namely, urobilinogen, urobilin and bilirubin; the tests for these substances will therefore be given together with special data bearing upon their individual significance.

There is, however, another way by which the total power of the liver to carry on its external secretory function may be judged. This consists theoretically, in finding a substance which if injected into the circulation will be eliminated exclusively by the liver. It was only in recent years that such a substance was discovered, phenoltetrachlorphthalein.

Tests to determine the status of the excretory functional power of the liver may, therefore, be properly divided into two categories:

1. Tests for urobilinogen, urobilin and bilirubin in the urine with the interpretation of results in reference to hepatic insufficiency. 2. Tests to determine the global capacity of the liver to eliminate foreign substances. In this category there is but one test so far devised, namely that of phenoltetrachlorphthalein.

1. Tests for Urobilinogen, Urobilin and Bilirubin in the Urine. Interpretation of Results with Reference to Hepatic Insufficiency

The Urobilinogen Test. Ehrlich's Test.[34]—*The Benzaldehyde Reaction.*—While investigating the aniline dyes for their effects upon trypanosomes, Ehrlich found that the addition of paradimethylamidobenzaldehyde to certain fresh urines produced a bright red coloration. This was in 1901. In 1903 Pappenheim [35] called attention to the fact that the reaction occurred only in those urines which, on standing, gave the reaction of urobilin. In the same year Neubauer [36] demonstrated that the reaction was due to urobilinogen, a colorless chromogen which gradually becomes converted into urobilin.

Technic.—The test is very simple. Add to fresh urine in a test tube several drops of Ehrlich's reagent. The reagent is as follows:

Paradimethylaminobenzaldehyde ...	8 gms.
Concentrated hydrochloric acid	80 gms.
Distilled water	200 gms.

If urobilinogen is present a red color appears. The color reaction in the cold urine is of pathological sig-

[34] Wien. med. Wchnschr., 1901, XV; Münch. med. Wchnschr., 1901, XV.
[35] Berl. klin. Wchnschr., 1903, II, p. 42.
[36] Sitz. d. Gesell. f. Morph. u. Physiol., Munich, 1903, July, H. ii.

nificance only when a distinct scarlet color is obtained.

There are a few sources of possible error. The ingestion of hexamethylamine or antipyrine may cause the same reaction. The presence of acetone in the urine must also be excluded as it produces a similar coloration.

If the reaction persists after free purgation it is more significant. The reaction is not constant in all conditions in which it has been found because the liver may be able to excrete enough urobilinogen to prevent its appearance in the blood and urine.

Urobilinogen has been found in the urine in many pathological conditions, chief of which are cirrhosis of the liver, cholangitis, infectious diseases, heart diseases in the stage of decompensation, pernicious anæmia, pulmonary infarction and visceral hemorrhage.

Ehrlich's test has been highly recommended as a clinical method for determining hepatic insufficiency by Müller, Bauer, Neubauer, Hilderbrand and others. It has been regarded by some observers as an adequate and infallible criterion of hepatic function. Some observers have claimed for it a prognostic significance. All of these contentions have been found to overstate the facts.

The presence of urobilinogen in considerable quantity in the urine may indicate that there is a partial interruption in the biliary excretion and that some of the intestinal urobilin absorbed into the blood is not being thrown off by the liver. The primary cause may be either a disorder of the liver cells or a congestion or toxemic obstruction of the biliary channels.

The discovery of a persistent urobilinogenuria should therefore point to a careful study of the hepatic functions by all known means. If hepatic insufficiency can be ruled out the only remaining explanation is that excessive destruction of the blood corpuscles is taking

place somewhere in the body.

Recently rather strong criticisms of Ehrlich's test have appeared. Wilbur and Addis [37] in 1913 stated that they do not believe it constitutes a reliable criterion of hepatic function. This opinion has been reiterated by Chesney, Marshall, and Rowntree,[38] last year. All these authors maintain that observations on a single or 24-hour specimen of urine have no significance. That great variations appear from day to day and that only when repeated tests are made covering a period of two weeks, controlled by studies of urobilin content in the feces, can the results be accepted.

The simplicity of Ehrlich's test will insure for it a permanent, if not paramount, place. It must always be remembered that only a persistent and well-marked urobilinogenuria is to be regarded clinically as significant.

A well-marked and lasting reaction appears to indicate one of two things, an hepatic insufficiency or excessive hemolysis. There is nothing in the test itself to enable a differentiation between the two to be made. The test is therefore of some importance when regarded only as a corroborative sign of insufficiency of the excretory function of the liver.

Tests for Urobilin.—First add to the urine a few drops of 10% solution of zinc chloride and enough ammonia to dissolve the precipitate. Filter into a test tube and hold same against a dark background; a green fluorescence denotes urobilin. Equal quantities of 1% solution of zinc acetate and urine may be used in the first part of the test. The fluorescence may be made more visible by concentrating light on the tube with a

[37] Arch. of Int. Med., 1913, Feb., p. 235.
[38] Jour. Amer. Med. Assn., 1914, LXXIII, p. 1533.

lens.

Another and perhaps more delicate method of detecting urobilin is to extract 50 c.c. of urine with 50 c.c. of pure ether. Pour off the ether into a tube and evaporate. Dissolve the brown residue in a little strong alcohol. The solution will be pale yellow with a green fluorescence if urobilin is present.

A third method is to acidify 20 c.c. of urine with several drops of HCl. Shake gently with 5 c.c. of amyl alcohol. This extracts the pigment and shows a bright green fluorescence when treated with an alcoholic solution of zinc chloride and a little ammonia. By transmitted light the amyl alcohol is a faint pink shade.

The most rapid method of testing for urobilin is by the use of the pocket spectroscope. If the urine is dark it should be diluted. The characteristic spectrum band is seen between the green and blue, between the lines C and F.

Tests for Bilirubin.—The tests usually employed are those of Gmelin, Rosenbach, Huppert and Smith.

Gmelin's Test.—The urine is treated with sufficient concentrated HNO_3 containing a trace of nitrous acid, sufficient to form a layer beneath. If bilirubin is present there will be a play of colors at the zone of contact from yellow through green, blue, violet, red and orange. The green will lie nearest the urine and the orange in the upper acid.

Rosenbach's Test.—Filter the urine through Swedish filter paper; apply a drop of HNO_3 containing a trace of nitrous acid upon the paper. The play of colors above mentioned under Gmelin's test will appear.

Huppert's Test.—Precipitate the urine with barium chloride and ammonia. The precipitate is washed with water. Wash the precipitate with alcohol into alcohol

acidulated with sulphuric acid. Boil for a time. If bilirubin is present an emerald green color will appear.

Smith's Test.—A small amount of urine is placed in a test tube and overlaid with a few c.c. of tincture of iodine diluted with alcohol 1:10. If bilirubin is present a distinct emerald green ring will develop at the zone of contact.

2. Tests to Determine the Global Capacity of the Liver to Eliminate Foreign Substances

The Phenoltetrachlorphthalein Test of Liver Function. Rowntree, Horwitz, and Bloomfield Test.—Phenoltetrachlorphthalein was originally studied pharmacologically by Abel and Rowntree [39] in 1909. It was proposed in 1913 as a test for functional capacity of the liver by Rowntree, Horwitz, and Bloomfield. (Johns Hopk. Hosp. Bull., 1913, XXIV, p. 327.) Important experimental work was done on the drug in 1913 to determine its behaviour in different liver injuries, by Whipple, Mason and Peightal,[40] and by Whipple, Peightal and Clark.[41]

Great interest is attached to the phenoltetrachlorphthalein test for liver function; first, because it bids fair to become the most satisfactory test for the purpose yet devised, and, secondly, because all the work upon it has been done in America.

Professors Orndorff and Black [42] of Cornell University were the first to make the substance in 1909. The pharmacological investigations of Abel and Rowntree included a study of phenolphthalein together with the new synthetic phthaleins, with special reference to their

[39] Jour. Pharmacol. and Exper. Therap., 1909, I, p. 231.
[40] Johns Hopk. Bull., 1913, XXIV, p. 207.
[41] Johns Hopk. Bull., 1913, XXIV, p. 343.
[42] Amer. Chem. Jour., XLI, 1909, p. 349.

behaviour as purgatives. They found that phenolphthalein and its halogen substitution products, of which phenoltetrachlorphthalein is one, do not differ greatly in their physiological action. They are nonirritant to mucous membranes and subcutaneously when injected in oil. They are of low toxicity and possess no bactericidal action.

Both phthaleins are laxative when given by mouth, subcutaneously or intravenously. When an oily solution of phenoltetrachlorphthalein (.4 gm.) is injected under the skin of dogs or human beings a laxative action is induced which continues from 4 to 6 days. When the tetrachlorphthalein is given subcutaneously it escapes from the body exclusively in the bile. When the same substance is given by mouth it is not absorbed. After subcutaneous administration of phenoltetrachlorphthalein the drug escapes dissolved in the bile and becomes later absorbed by the mucous membrane of the large intestine.

These facts form the physiological basis for the phenoltetrachlorphthalein test. The drug is eliminated exclusively, or practically so, in the bile, and since this excretion can be hurried through by purgatives, no time will be given for its absorption, and thus the actual amount of the drug eliminated may be found.

The experimental work done by Whipple, Mason and Peightal, and by Whipple, Peightal and Clark has established the fact that there is a striking parallelism in animal observations between the amount of experimental liver injury produced and the amount of phthalein eliminated.

Their method of determining this fact was as follows: Female dogs were used because of ease of catheterization. The dogs were given intravenously .1 gm. of the phthalein when weighing between 10-20 pounds; .2 gm.

when weighing over 20 pounds. The injection was given in the forenoon and 200-300 c.c. of water administered by stomach tube. After 5 or 6 hours the urine was collected and magnesium sulphate and croton oil given to produce several semi-fluid stools. The feces were collected next morning. The total feces were then diluted to 1 to 2 liters. The mixture was made alkaline with 5-10 c.c. of 40% solution of sodium hydroxide and shaken until uniform. One tenth of this quantity was taken and diluted to 500 c.c. with water, 3 to 4 c.c. of 40% solution sodium hydroxide added and the mixture thoroughly shaken. Of this second solution 100 c.c. were precipitated with 5 c.c. of saturated solution of basic lead acetate. After a few seconds a curdy precipitate fell. The solution was made up to 200 c.c. with water containing 4 c.c. of 40% solution sodium hydroxide. On standing, the supernatant fluid showed a clear phthalein color. This was filtered and the clear solution read off in a colorimeter against a standard solution .01 gm. phthalein to the liter.

In the hands of its authors, this method gave pretty uniform results; on normal dogs the amount excreted being 35 to 50% with .1 gm. injection and 40 to 50% with .2 gm. injection. The drug did not appear in the urine. About 10-15% of the phthalein injected was lost from the time of the injection to that of its being poured out by the bile into the intestine. This shows that the liver is quite specifically concerned in the elimination of the substance, phenoltetrachlorphthalein.

Whipple and his collaborators found that when the liver parenchyma is artificially injured, as by chloroform, phosphorus or hydrazine, there is a very notable drop in the output of the substance in the feces down to 20 or 10% or even a mere trace. The phthalein then begins to be excreted by the urine. When the hepatic

lesion improves, the phthalein output in the bile increases toward normal. The normal output of phenoltetrachlorphthalein in dogs is 45% of the amount injected. The drop in phthalein output is always proportional to the extent of liver injury. If the injury is grave enough to produce death, the phthalein output falls to zero. In some instances as the effects of the hepatic injury are spontaneously repaired, there may be an actual increase of phthalein output even above normal, a hypersecretion of phthalein as it were.

Besides injury to the liver parenchyma by poisons, the above investigators found that severe circulatory disturbances artificially produced are followed by a drop in the phthalein output. Actual destruction of liver parenchyma produces similar results.

In experimental obstructive icterus there is, of course, no phthalein output, since none of the drug reaches the intestine. In experimental hematogenous icterus there is no modification of phthalein output.

The experimental studies so far performed indicate that the phenoltetrachlorphthalein will be valuable from a quantitative as well as a qualitative standpoint in the estimation of insufficiencies of liver function.

The clinical application of phenoltetrachlorphthalein as a test for hepatic function was first worked out by Rowntree, Horwitz and Bloomfield.

We owe to Rowntree the suggestion that the specificity of the liver in excreting phenoltetrachlorphthalein analogous to that of the kidney towards phenolsulphonphthalein would indicate that estimations in man of the quantity of dye excreted by the liver after an intravenous injection ought to afford a practical clinical method of determining the functional capacity of the liver.

It will thus be seen that the phenoltetrachlorphthalein

test of Rowntree, Horwitz and Bloomfield is founded upon rational theoretical considerations and that it was subjected to a very rigid experimental investigation.

Technic of Phenoltetrachlorphthalein Test. — An aqueous solution of the disodium salt is used. It is prepared by placing 2.5 gms. of phenoltetrachlorphthalein in a 200 c.c. Erlenmeyer flask with 5 c.c. of $^2/n$ NaOH solution and 45 c.c. of freshly distilled water. This is boiled under a reflux condenser for 20 minutes. The solution is filtered into a 100 c.c. flask. This solution is of 5% strength and is approximately isotonic with blood. It is intensely purplish red in color. Since the phthalein is precipitated by CO_2 in the atmosphere it will not keep more than a few days, hence requires to be freshly prepared for use. The patient is given two compound cathartic pills the night before the test is applied.

In making the test 8 c.c. of the solution which will contain about 400 milligrams of tetrachlorphthalein are measured. It has been found that this amount is never followed in normal persons with the excretion of any dye in the urine and is sufficient to produce an intense color in the final preparation of the feces. The 8 c.c. is given intravenously as follows, of course under strict aseptic precautions:

A funnel with properly connected intravenous system is filled with freshly distilled water or salt solution and the flow into the vein is started. When this is well established, the phthalein solution is added. Fifty to 100 c.c. of water are used and the phthalein solution is washed in with freshly distilled water until the fluid entering the vein is clear. About a quarter of an hour is required for the injection.

After the injection, the patient is given another pur-

gative, usually two compound cathartic pills, and this dose is repeated the following morning if the bowels are not running freely. The stools are collected for 48 hours in a covered vessel. The urine is collected for 24 hours.

The quantitative determination of the amount of phthalein passed is made as follows: The total feces collected are put in a wide mouth 2 liter bottle diluted with water to 1 or 1.5 liters, according to quantity, and the whole put in a shaking machine and well agitated for from 5 to 20 minutes.

One-tenth of the total amount is immediately poured off in a 1-liter flask. To this is added 5 c.c. of 40% NaOH, which makes the mixture a dirty red color. The flask is stoppered and thoroughly shaken. One hundred (100) c.c. of the contents of this flask are placed in a 200 c.c. flask, to which is added 5 c.c. of saturated basic lead acetate. This decolorizes the mixture and throws down a heavy precipitate which leaves a colorless supernatant liquid. Five (5) c.c. of 40% solution of NaOH are added, which produces the phthalein color. More hydroxide solution may perhaps be needed to bring out the full color, but excess should not be used. The contents of the flask are then made up to 200 c.c., shaken, and the solution allowed to stand five minutes. The supernatant fluid is clear and some can be poured off for colorimetric examination. The reading is made in a colorimeter similar to the one used in testing kidney permeability with phenolsulphonphthalein. The comparison solution is made by taking .4 c.c. of the original solution used for injection and diluting it up to 1 liter plus sufficient NaOH to make the deepest color. The per cent. of dye eliminated can be read off on the instrument.

If the reading is low be sure that the maximum color

has been developed by adding a little NaOH again to the 200 c.c. dilution above mentioned. NaOH must be carefully added because excess will tend to render solutions yellowish red instead of pure purple red.

In case the quality of color is unsatisfactory the authors of the test recommend the following procedure: After the addition of about 10 c.c. of 40% NaOH dilute the feces mixture up to 1 liter. Take one-tenth of this and add 5 c.c. of sodium hydroxide and water up to a liter. One hundred c.c. of this is put in a 200 c.c. flask and to it is added 5-10 c.c. or more of the following solution:

$CaCl_2$	90 gms.
Conc. NH_4OH	10 c.c.
Water	50 c.c.

This brings out a good quality of color. Dilute up to 200 c.c. and allow to stand covered for some hours, perhaps even 24. The supernatant liquid is then tested in the colorimeter with the standard solution as above.

The lower limit of normal output is 30%.

A year's experience with the phenoltetrachlorphthalein test of liver function has prompted a recent communication from Chesney, Marshall, and Rowntree (Jour. Amer. Med. Assn., 1914, LXIII, p. 1533) in which these authors conclude that outspoken changes in the liver can, in most cases, be demonstrated by the test. It was found positive in advanced cirrhosis, in passive congestion (cardiac liver) and in cancer and syphilis involving the liver.

These authors recommend the association with the phthalein test, of estimation of nitrogen partition in the blood and urine, and fibrinogen estimation in the blood serum.

They make the important general observation that the information to be derived from tests of the liver function does not compare in reliability with that applied to the kidney. Similar views have likewise been expressed by other writers. The reason would appear to be plain. We do not as yet understand the functions of the liver regarded as a unit or dissociatively as we do those of the kidney. The symptomatology of hepatic insufficiency is not understood to an equal extent with that of the kidney. However this may be, sufficient progress has been made to afford ample congratulations for the work of the past and an optimistic outlook for future developments.

Krumbhar [43] has recently stated as a result of his researches and investigations that the phenoltetrachlorphthalein test of Rowntree, Horwitz, and Bloomfield promises a greater value than all other tests so far devised for estimating the functional capacity of the liver.

[43] N. Y. Med. Jour., 1914, c. 719.

CHAPTER II

TESTS OF KIDNEY FUNCTION

GENERAL CONSIDERATIONS

Historical.—In 1830 Hahn noticed after ingestion of turpentine in gouty persons that the substance failed to render the urine odorous as it was known to do in healthy persons. In 1837 Rayer noticed the same thing with regard to asparagus.

Clinicians for a long time have known that many persons with nephritis can not take mercury, salicylates, iodides, bromides and various other drugs without rapidly showing signs of intolerance. Todd wrote upon this subject in 1857 and Roberts in 1865. Duckworth and Bouchard in 1873 showed experimentally that many drugs which normally pass quite readily through the kidney, fail to do so in nephritis.

The first practical application of these facts was made by Achard and Castaigne, who introduced methylene blue in 1897 as a direct test of the functional capacity of the kidneys.

Physiological.—As well expressed by Blum the fundamental function of the kidney is its osmoregulatory power; its power to constantly maintain at an unvarying point the molecular concentration of the blood. This it does by removing a series of substances whose accumulation in the organism would eventually

produce serious and even fatal consequences. These substances are removed in the urine.

Two general theories of urinary secretion have existed, side by side, for several decades. They are known eponymically by their originators and although they have been added to or subtracted from in details by a host of subsequent workers they yet stand as opposing schools of physiological interpretation. These schools are known as that of Ludwig on the one hand and that of Bowman-Heidenhain on the other.

According to Ludwig, the elimination of urine is a simple process of physical filtration and diffusion. The anatomical structure of the glomerulus and the physiological conditions existing therein, appearing to favor the idea of filtration, Ludwig believed that water passes through the epithelium of the capillary wall and the glomerular epithelium as through a filter, carrying with it sodium chloride and other inorganic salts and urea, and that the diluted urine in its passage through the uriniferous tubules becomes concentrated through loss of water by diffusion into the more concentrated blood and lymph.

According to the other theory, that of Bowman-Heidenhain, the elimination of urine is fundamentally a secretory act and not fundamentally a physical act. It assumes that the glomerulus secretes water and inorganic salts while the epithelial cells of the uriniferous tubules secrete urea and the other specific constituents of the urine.

We shall not attempt here either a historical or physiological review of these theories. It may be said that the majority of physiologists adhere to the more conservative and vitalistic hypothesis of Bowman-Heidenhain. The grounds for this belief, and, indeed, all of the facts bearing upon both sides of this now classical

controversy, are properly to be found in any modern text book of physiology.[1]

It is pretty generally agreed that whether by filtration or secretion, water leaves the kidney through the glomerulus. Beyond this generally accepted fact there is so little unanimity of opinion as to the exact place where sodium chloride urea and the other solid constituents of the urine are eliminated, that it is impossible to make any categorical statement with reference thereto. Many attempts have been made to divide up the total kidney function into categories or topical functions and to locate these functions anatomically in parts of the glomerulo-tubal structure. But it cannot be pretended at the present time that any such differentiation has been proven, certainly not to such an extent as to justify deductions of great practical importance. This question will, however, be more fully elaborated later.

The composition of the urine is far from simple. Its chief constituents, apparently, are water, sodium chloride and urea. But besides these substances, urine contains purine bodies (uric acid, xanthin, hypoxanthin), creatinin, oxalates, glycuronates, phosphates, sulphates, various oxy-nitrogenous and fatty acids, and the pigments urochrome and urobilin.

Nearly all of the nitrogen excreted from the body is supposed to pass through the kidney. The total amount of nitrogen eliminated in the urine in 24 hours is consequently regarded as the most important index of proteid metabolism. The actual estimate of total urinary nitrogen is usually done by the method of Kjeldahl (described under Liver Tests, *q. v.*).

The total weight of nitrogen in the urine multiplied by 6.25 gives the amount of protein broken down in the body, since nitrogen forms 16% of the weight of

[1] See Howell, Halliburton, etc.

the protein molecule. The total amount of nitrogen eliminated in 24 hours by a normal adult is between 14 and 18 grams, which corresponds to 88-117 grams of protein.

The total nitrogen eliminated in the urine is divided as follows: 1. Urea nitrogen; this averages 87.5% of the total. 2. Ammonia nitrogen; this averages 4.3% of the total. 3. Creatinin nitrogen, 3.6%. 4. Purin nitrogen, variable.

Urea occurs in the urine as its chief nitrogenous constituent (about 2%). Since a normal adult secretes 1500 to 1700 c.c. of urine in 24 hours the amount of urea eliminated will vary from 30 to 34 grams. Urea, of course, is not manufactured by the kidneys, but is merely eliminated by them. Urea is a normal constituent of blood existing in that fluid in quantities varying from .035 to .153%. If the kidneys are removed or become impassible to urea this substance accumulates in the blood.

Sodium chloride is the chief inorganic constituent of urine, amounting to about 15 grams per day in a normal adult.

Under pathological conditions a variety of substances organic and inorganic may appear in the urine, whose search and identification is a part of the routine analysis conducted for clinical purposes.

A consideration of these substances belongs, of course, to the domain of general clinical pathology. The question of how far an ordinary urinary examination can serve to reveal the functional capacity of the kidney will be taken up presently.

The kidney functionates normally at a point below its maximum capacity, retaining unused a certain amount of functional energy which constitutes its reserve. When this reserve becomes exhausted the func-

tional capacity of the organ will be irremediably damaged unless it can recuperate. When uremia or edema have appeared the amount of functional incapacity of the kidney has become considerable. No special test of function is required to discover it, perhaps, but even under such conditions, it may be extremely useful to determine just how far, in any given case, the depreciation of functional integrity has gone.

Classification.—There are various general plans by which the functional state of the kidney may be investigated.

In the first place we may determine how far the kidney is able to eliminate increased amounts of its normal constituents, such as water, salt and urea.

In the second place we may select substances foreign to the organism, but which are eliminated by the kidney, and determine the rate and quantity of their excretion. Iodide of potassium, lactose, phenolsulphonphthalein, phloridzin are examples of such substances.

Thirdly, the study of the blood will constitute another avenue of approach to the problem of estimating the function of the kidney, because one important result of renal insufficiency will be the accumulation of substances in the blood which should be eliminated in the urine. Among methods of this type may be mentioned partitive estimations of nitrogen in the blood, particularly incoagulable nitrogen. Such examinations will often disclose an abnormal degree of accumulation or retention of such products in the blood if a condition of renal impermeability or insufficiency exists.

Thus it will be seen that all tests for kidney function are based upon the broad principle that any depreciation of renal activity will be reflected in the urine on the one hand and the blood upon the other. The urine will contain less of its normal constituents than normally

and less of any substance artificially eliminated by the kidney, while the blood will show the effects of renal inadequacy by disclosing an accumulation in the plasma of substances which are normally excreted continuously in adequate amounts.

All tests for renal function so far devised may be satisfactorily divided into the three following categories:

1. The urine as an index of renal function—(a) Urinalysis, (b) Physical and Biological characteristics. 2. The Blood as an index of renal function. 3. Elimination of foreign substances by the kidney as an index of renal function.

In the following synopsis, we may see how the various tests which have come to be used can be distributed among the three classes:

I. Urinalysis as an index of renal function.

A. Urinalysis.
1. Estimation of water: experimental polyuria.
2. Estimation of sodium chloride: experimental chloruria.
3. Estimation of urinary nitrogen; urea, etc.
4. Test meal for nephritic function.
5. Estimation of urinary coloring matter.
6. Estimation of urinary diastase.

B. Physical and biological characteristics.
1. Cryoscopy of the urine.
2. Electrical conductivity of the urine.
3. Estimation of urinary toxicity.

II. Studies of the blood as indices of renal function.
1. Estimation of blood urea and of incoagulable (residual) nitrogen in blood.

2. Ambard's coefficient and the index of urea excretion (McLean).
3. The concentration of creatinin and uric acid in the blood.
4. Estimation of coagulation time.
5. Cryoscopy of the blood.

III. Studies of elimination of foreign substances by the kidney as criteria of function.

1. Miscellaneous Chemical Substances.
   a. Potassium Iodide.
   b. Phloridzin.
   c. Hippuric Acid.
   d. Lactose.
2. Dyes or colors: experimental urinary chromoscopy.
   a. Methylene blue.
   b. Indigo carmine.
   c. Phenolsulphonephthalein.

I. THE STUDY OF URINALYSIS AS AN INDEX OF RENAL FUNCTION

A. Urinalysis

The urine represents, as it were, the concrete results, almost the total results of renal activity. Inasmuch as the entire urinary output of the kidney for any given period of time may be readily collected it would seem natural to assume that a chemical analysis would throw all the light that is necessary upon the problem of renal function; but as a matter of fact, while it is true that chemical analysis of the urine provides an adequate insight into the amount of salts, of water and of urea secreted by the kidney, it is not true that urinalyses

are sufficient to determine the functional capacity of the organ. Gross anatomical and physiological disturbances are often thus discovered and extremely important information is thus derived concerning the diagnosis of diseases of the kidney and urinary organs.

But this is a very different proposition from determining thereby the functional capacity or incapacity of the kidney, in the absence of evidence of gross organic lesion. Even under apparently normal circumstances the actual amount of the different urinary constituents excreted may vary considerably. For example the chlorides may be eliminated in excess and nitrogen retained or vice versa. But such variations are not necessarily dependent on anatomical lesions of the kidney or even upon any disturbance of renal permeability.

If, in a given 24-hour specimen of urine, the figures representing the elimination of the different important constituents depart from the usual normal, we cannot draw any absolute conclusions from this fact alone as to whether the functional capacity of the kidney is below or above normal. One reason for this is that the chemical constitution of the urine is not dependent alone upon kidney functional power but it is influenced by a large number of extremely important extrarenal factors, among which may be mentioned the intake of food and fluids, the condition of the nervous system and other organs, etc.

It is a well-known fact that before any important conclusions as to nitrogen metabolism can be drawn from the chemical constitution of the urine, these factors must be taken into consideration, and if they are adequately considered the task becomes complicated by many necessary experimental refinements. The whole question of body metabolism must be taken up. The intake and output in every direction must be measured.

But even after this is done and it is demonstrated from urinalysis that there is a deficit in nitrogen excretion, the kidney function may be perfect and the nitrogen simply retained in the tissues.

Variations in nitrogen elimination occur under so many different conditions that interpretation is often difficult or impossible. The same may be said with regard to the output of other constituents of the urine.

Concerning the application of urinalysis to the interpretation of kidney function it may be said that if the figures are all consistently and invariably normal, the kidney function is apt to be good; and if there is a persistent and considerable departure from normal the kidney function may be deficient.

But in order to be of real value, the tests, particularly those regarding the nitrogen excretion, must often assume the proportion of metabolism experiments, which makes them impractical for clinical use.

Fortunately for the purposes of clinical medicine the physician will not be called upon to consider the intricate problems of nitrogen metabolism in his investigations of renal function. His desire will be to know the capacity of the kidney to do its work and fortunately this object may be accomplished without recourse to extremely elaborate and technical processes.

We are justified in expecting that under an average regimen the kidneys will eliminate somewhere near the average amounts of the urinary constituents. But knowing how many and how variable the extrarenal factors are which influence the absolute quantities of urinary constituents eliminated, it will become apparent that the results of a urinalysis, no matter how complete, will require to be supplemented by other and better means of determining the functional capacity of the kidney.

1. Estimation of Urinary Water as an Index of Renal Function. Experimental Polyuria. Albarran's Method

The healthy kidney possesses the power in a high degree to adapt itself to those tendencies, such as addition or subtraction of water to or from the circulating blood, which would tend to alter the molecular concentration of the fluid. It quickly re-establishes both molecular and water equilibrium, thus maintaining an equable osmotic tension in the blood and lymph. Superfluous water is, normally, quickly eliminated through the glomerulus and reabsorption in the canaliculus is inhibited. When the supply of water to the organism is deficient, the resorptive function of the canaliculus is raised and a more highly concentrated urine is eliminated. This function of the kidney may be properly termed its diluting-concentrating power.

Since water secretion is a function of the glomerulus, the diluting power of the kidney is a glomerular function. The functionally weak kidney is not only unable to produce a highly concentrated urine but also unable to elaborate a much diluted one. The concentrating power of the kidney is a function of the epithelium of the uriniferous tubes (canaliculus).

The diluting-concentrating power of the kidney suffers in diffuse kidney disease in proportion to the amount of parenchyma involved. In parenchymatous nephritis the water secreting power of the kidney is lowered; in contracted kidney it is more or less retained.

The increased urine following an experimental provocative polyuria test differs from the increased secretion following a heavy meal, since in the former case the freezing point (Δ), molecular concentration, chloride, phosphate and urea content (specific gravity) are all diminished, while in the latter they are all increased.

With respect to continuity of function the diseased organ possesses a greater constancy and invariability in proportion to the amount of disease. The normal kidney function tends to vary, that is, to adapt itself to the constantly changing conditions in the organism. The diseased organ has no such power.

The healthy kidney always functions below its maximum strength; always possesses, in other words, a certain reserve power which can be used under extraordinary circumstances, such as great increase in water and solid molecular intake. The insufficient kidney is unable to meet these requisitions for added energy, and responds but little if at all to extra stimulation. It has lost its reserve.

As a corollary to the above it may be added that if one kidney is diseased and the excretion from the two organs be compared, the facts as above stated will apply. The affected kidney is less able to respond to adaptation requirements than the normal and the degree of its failure to do so may properly be taken as the measure of its incapacity.

For these reasons polyuria tests may be employed with the view of conducting examinations upon the total excretion or whenever necessary upon the excretion obtained by ureteral catheterization from each organ separately.

The Water Tests.—The provocative polyuria tests are usually carried out with water. The tests should be applied in the morning on an empty stomach. The morning urine prior to the test should be measured and examined for quantity and specific gravity, total sodium chloride and urea elimination and perhaps cryoscopically. The patient is then given 500-700 c.c. of mineral water or ordinary water. The urine should be collected every half hour by voiding or catheter if the

total amount is to be examined (general renal function) or by ureteral catheterization if the separate kidney functions are to be compared.

Under normal circumstances the polyuria appears within the first half hour, reaching its maximum at this time, and quickly sinking. The content of solids sinks. The freezing point (△) diminishes.

If the functional power of the organ is below normal, the polyuria is delayed or does not occur and the amount of variation from the normal may be taken as a fair measure of the incapacity.

Straus-Grünwald Method.—The patient takes nothing after 7 P. M. into the stomach. At 6:30 A. M. the following morning a pint of water is ingested. The night urine is collected; also that voided at 7, 8, 9, 10, and 11 A. M. The amount and specific gravity of each portion are recorded. The patient remains in a reclining position during the time of the test.

In normal cases an amount of urine is passed in the first 3 hours equal to that which was drank. That is by 10 A. M. at least a pint is voided. At 8 A. M. the sp. gr. is lowest. Variations in the amount voided, time required, and specific gravity will indicate abnormal renal function.

The Diuretic Tests (pharmacological).—Caffein, diuretin, theophyllin (theocin), euphyllin and other diuretic substances have been employed, but none of these drugs has been found to possess much advantage over the simple water test. The diuretic drugs appear to increase the solid constituents of the urine as well as the fluids. Blum, who introduced euphyllin, does not recommend it because he has found a fall of blood pressure follow its hypodermic or intravenous administra-

tion.

None of these tests have acquired a sufficient prominence to justify their description.

While the so-called water or polyuria tests have proven of some value in estimating relative function in the separate kidney secretions, it is generally agreed that they are of much less importance in estimating total kidney function.

The absolute quantity of urine voided varies very greatly under normal and abnormal circumstances. According to Rowntree and Fitz there is no constant relation between the existence of polyuria or oliguria and the condition of kidney function as shown by other well-recognized tests. This applies to nephritic and cardiac cases and to combinations of these.

The specific gravity of the urine in advanced nephritis according to these authors is usually low.

2. Estimation of Sodium Chloride as an Index of Renal Function

Ten to fifteen grams of sodium chloride are excreted by a normal adult in 24 hours. The rapidity with which the kidneys can excrete a considerable amount of sodium chloride has been suggested and employed as a test for renal function.

With regard to the method of so-called forced elimination of sodium chloride, it must be freely granted that the problem is a very difficult and complicated one.

If diminished secretion of sodium chloride always indicated renal impermeability the problem would be solved, but this is by no means the case. The tissue fluids themselves, everywhere in the body perhaps, have varying affinities for sodium chloride and a diminution of chloride elimination may not signify a diminished

permeability of the kidney for salt but only an increased retention of salt in the body.

Nevertheless there is, under normal circumstances, a very close relation between the intake of sodium chloride in the food and its elimination by the kidneys. So that if an individual is placed for a period of time upon a regimen containing a low percentage of salt, the excretion of that substance will become reduced to a lower equilibrium. If now the quantity of sodium chloride ingested be suddenly increased there will be an immediate and proportionate increase in the amount secreted.

If there is no increase, it will be difficult to determine whether there is chloride retention or defective excretion so that the chloride test is seldom used alone as a measure of renal function.

The exact situation in the glomerulo-tubular mechanism where sodium chloride is excreted, is not known with certainty. According to the rather recent investigations of Schlayer and Takayasu and Von Monakow, sodium chloride is excreted by the tubular epithelium, or more exactly the excretion of salt following its administration in amounts in excess of the usual daily intake is accomplished by the tubules.

When large amounts of salt are ingested the excretion, according to Schlayer, takes place in one of two ways, depending upon the amount of water simultaneously absorbed. If the salt is given without extra water it is almost entirely secreted within 24 hours without diuresis, by an increased concentration of the urine. If, however, it is given with an excess of water it is secreted partially through increased concentration and partially through diuresis.

If the vascular structure of the kidney is injured, the ingestion of salt may be followed by marked diuresis, the salt all escaping in the urine in 24 hours with-

out the percentage content being increased. The specific gravity may be low and tends to remain at a fixed point. To this combination of phenomena, Schlayer gave the name vascular hyposthenuria, and, according to his idea, the inability of the kidney to eliminate a urine concentrated in salt is not due to tubular defect but to a hypersensitive condition of the blood vessels which allows the secretion of the salt in relatively large amounts of water; in other words, the sensitive vessels respond to the salt administration by the diuresis. When the vascular injury is more marked the vessels do not so react but respond with oliguria.

In severe tubular epithelial disease, however, the quantity of salt eliminated is not raised by the administration of salt. Here a urine of fixed low specific gravity of moderate quantity is obtained. The salt is retained. There is, according to Schlayer, a tubular hyposthenuria.

These interesting findings reported by Schlayer and his school have not been completely corroborated and it does not appear to be agreed at the present time that any absolute distinction between vascular and tubular hyposthenuria can be founded upon the response of the kidney to tests with sodium chloride.

In fact the number of extrarenal factors concerned in the salt output are so many and so illy understood that the salt test alone is considered of no value. But when considered in conjunction with other functional tests and with clinical findings in cardiac and renal cases it may be of some diagnostic and prognostic value.

In advanced nephitis there seems no doubt that salt elimination is lesened to a certain extent but if the patient has been on a salt-poor diet for a time previous to the test, the tissues will retain salt when

administered regardless of the cardiorenal condition.

Œdema is the symptom in chronic nephritis which is usually associated with the idea of chloride retention. It is not positively known just what factors are concerned in this, or whether they are chiefly renal or extrarenal.

It is on the basis of the supposed connection between œdema and chloride retention that the now well-known method of salt reduction in the treatment of œdema in Bright's disease was introduced.

Technic of Sodium Chloride Test. Test of Alimentary Chloruria.—The patient is placed on a diet containing about 5 gms. of salt per day. After several days or when the salt output is approximately constant, 5-10 gms. of sodium chloride are given, dissolved in 125 c.c. of water. The quantity is taken in three portions during the day. This is kept up for four consecutive days. The daily output of chloride is determined by the method described below. If the patient is kept on a salt-poor diet, say 2.5 gms. daily for some time previous to the test, it will be found that excretion will always be lessened from a normal tendency of the tissues to retain chlorides. For this reason the best method consists in merely establishing chloride equilibrium before the test is started.

Estimation of Sodium Chloride in the Urine.—The principle of the test is that chlorides are precipitated by solutions of nitrate of silver. Volhardt's method with its various modifications is regarded as the most accurate quantitative method of chloride estimation, and is used when the exact amount must be known as in metabolic experiments.

For all practical purposes Mohr's method will suf-

fice.[2] The strength of silver solution used in the test is such that 1 c.c. corresponds to .01 gm. of sodium chloride. Such a solution contains 29.06 gms. of pure fused silver nitrate to the liter. The technic of the estimation is as follows:

10 c.c. of urine previously freed from albumen are put in an Erlenmeyer flask or porcelain capsule and 100 c.c. of water added and several drops of potassium chromate solution, enough to produce a distinct yellow color.

The standard silver solution is added from a burette, stirring until the reddish orange color which appears first where the drop falls is distributed. The first permanent orange color trace is the end of the reaction. The operation should be repeated if necessary, to make certain of results. The number of c.c. used multiplied by .01 gives the amount of sodium chloride. The results are a little high but near enough for practical purposes. A rough estimate of the amount of chloride in the urine may be made as follows: To a test tube of clear urine non-albuminous, add 10 drops of pure HNO_3 and one drop of $AgNO_3$ (1 to 8). If chlorides are normal or increased, the precipitate is a compact ball which sinks to the bottom. If diminished, the ball is less compact. If much diminished, only a cloud is produced without solid flakes. This last represents a chloride content of 1% or less.

3. Estimation of Urinary Nitrogen as an Index of Renal Function

When functional tests of the liver were being discussed (*v.s.*) considerable emphasis was laid upon in-

[2] The Lutke-Martius method is often recommended, see Sahli's Diagnostic Methods, 1911, p. 455.

terpreting ureagenetic disturbance of that organ by taking into consideration the different phases of nitrogen metabolism which are so closely connected with liver activity. It was then shown that a knowledge of total nitrogen elimination in the urine and particularly of the different forms in which the nitrogen is eliminated, will shed light upon the condition of liver functional power.

The reasons for these assumptions were given in their proper place, but it may be serviceable to refer again to the fact that the liver is regarded as a very important locus for the synthesis of urea in the body. For this reason it is quite reasonable to suppose that functional depreciation of the liver cells would be reflected to an appreciable extent by the quantitative relative variations of the nitrogen constituents of the urine.

When now we come to the significance of nitrogen elimination in the urine to disorders of kidney activity, it will be necessary to remember that the kidney has nothing to do whatever in a specific way with the nitrogen metabolism. Its only function is to excrete the nitrogenous waste products which are brought to it by the blood. That urea is not manufactured by the kidney is proved by the fact that if blood is perfused through an isolated kidney, no urea is formed even tho substances such as ammonium carbonate, from which urea is readily produced, are added to the blood. It is well known that if the kidneys are removed in animals or their function paralyzed, urea will continue to accumulate in the blood as long as the animal survives.

No physiological fact is better known than that the relative amount of urea nitrogen in the urine varies directly with the amount of protein food ingested.

Other nitrogenous constituents of the urine, the purin bodies and creatinin, are unaffected by the intake. This suggested to Folin that most of the urea in the urine may come directly from food proteins which, having been hydrolyzed in the intestine into amino acids, are absorbed and further hydrolyzed and oxidized and the nitrogen constituent immediately eliminated as urea. The liver has most to do with this process though the urea forming function of this organ is known to be shared by some other tissues since even after the removal of the liver some urea is formed.

These physiological principles being agreed upon, it is easy to appreciate that the question of the estimation of urinary nitrogen as an index of renal function will be practically confined to the estimation of the amount of urea eliminated under normal circumstances, the patient being on a fixed diet, with an estimation of the power of the kidney to eliminate more urea when the proteid intake is increased, or when urea itself is ingested. The question of nitrogen accumulation in the blood as an index of renal insufficiency will be discussed below, when the study of the blood as an index of renal function is considered. It may be admitted here that the study of the partition of nitrogen and particularly quantitative estimations of urea and incoagulable nitrogen in the blood serum are of much greater significance in the estimation of kidney function than the same or related studies applied to the urine.

Diminished and Delayed Excretion of Urea.—This is an old criterion of functional renal power. The physiological principles upon which it is based have just been given. In order that this test of renal function shall be really conclusive, the patient must be put for some days upon a fixed regimen in which the amount of protein is definitely known.

The feces and urine must be examined to determine what part of the nitrogen has escaped in both. In carrying out such procedure, the experiment rises to the dignity of a metabolic investigation and requires great care and patience besides considerable technical skill. For this reason such a procedure cannot be regarded as adapted to clinical use.

Without the above precautions the value of ordinary routine urea estimation in the urine as a criterion of renal function is extremely doubtful.

If the dietary conditions can be reasonably controlled and a perfectly and persistently normal output of urea results, the renal function is at least equal to the ordinary demand of that person. If the proteid intake is increased and the urea excretion undergoes a corresponding and immediate rise, it may be concluded that the reserve force of the kidney is not materially damaged.

But negative results need not necessarily be taken to indicate a defect of renal function because of the fact that digestive disturbance and diminished liver function will cause the same thing to occur. If these extrarenal factors of error can be eliminated, then a diminished output of urea may become a valuable and reliable index of renal inadequacy.

Under a later chapter, concerning study of the blood as an index of renal function, it will be shown that the quantitative estimation of nitrogen partition in that fluid is a much more reliable test of renal functional power than urea estimations in the urine.

Repeating what has been given under general considerations it may be stated that a normal adult secretes from 30 to 34 grams of urea in the urine every 24 hours.

The simplest quantitative test for urea in urine is

that of Marshall, which has been described (*v. s.*). This method is so simple and so accurate that every clinician should familiarize himself with it.

Forced Urea Elimination. Provocative Urea Test of McKasky.—Technic. Thirty grams of urea dissolved in four to six ounces of water are given with a small breakfast, such as a cup of gruel. Follow this with four or five ounces of water to assist diuresis. The urine is collected every two hours for twenty-four hours, beginning two hours before the urea is given, so that a standard for comparison may be had, to determine the amount of increase. Quantitative determinations of urea are made in the different specimens at the end of the time.

The maximum excretion, its time, incidence, and the curve for the 24 hours, is thus determined.

Under normal conditions there is a sharp rise in urea excretion in the second two-hour period. When the kidney function is deficient the sharp rise is absent or is much delayed.

The only factor liable to disturb the interpretation of this test is gastric stasis.

Theoretically, the provocative urea test should be useful. There does not appear to be any reason why the urea could not be injected in smaller amounts, directly into the circulation, in which event the liability of misinterpretation through retention in the stomach would be avoided. The test has not been subjected to any clinical examination, so far as I know.

4. Test Meal for Nephritic Function

As a composite test for substances normally eliminated in the urine, and for the observation of the va-

riations in specific gravity, the test meal for nephritic function now occupies a place of prime importance. Hedinger and Schlayer * recently proposed a qualitative test of the mode of urinary function, as measured by the specific gravity, salt and water excretion in two-hourly periods. This method of study of kidney function has been especially amplified by the work of Mosenthal. His results have been corroborated by the writer and many other workers, and the following is from his observations and conclusions. The original name of "nephritic test meal" was recently changed to the "test meal for nephritic function" at the suggestion of L. F. Barker.

The following form contains the directions for the test meal:

Diet

TEST MEAL FOR NEPHRITIC FUNCTION

For ....Date ....

All food is to be *Salt Free* food from the kitchen.

Salt for each meal is to be furnished in weighted amounts. One capsule of salt, containing 2.3 gm. sodium chloride, is furnished with each meal. The salt which is not consumed is returned to the laboratory, where it is weighed, and the actual amount of salt taken calculated.

All food or fluid not taken must be weighed or measured after meals, and charted in the spaces below.

Allow no food or fluid of any kind except at meal times.

Note any mishaps or irregularities that occur in giving the diet or collecting the specimens.

Breakfast, 8 A. M.

Boiled oatmeal, 100 gm....................
Sugar, ½ teaspoonful....................
Milk, 30 c.c..........................
Two slices bread (30 gm. each)............
Butter, 20 gm........................
Coffee, 160 c.c......................
Sugar, 1 teaspoonful....................
Milk, 40 c.c.........................
Milk, 200 c.c........................
Water, 200 c.c.......................

Dinner, 12 Noon.

Meat soup, 180 c.c....................
Beefsteak, 100 gm....................
Potato (baked, mashed or boiled), 130 gm....
Green vegetables as desired...............
Two slices bread (30 gm. each)............
Butter, 20 gm........................
Tea, 180 c.c.........................
Sugar, 1 teaspoonful...................
Milk, 20 c.c.........................
Water, 250 c.c.......................
Pudding (tapioca or rice), 110 gm..........

Supper, 5 P. M...........................

Two eggs, cooked any style...............
Two slices bread (30 gm. each)...........
Butter, 20 gm........................
Tea, 180 c.c.........................
Sugar, 1 teaspoonful...................
Milk, 20 c.c.........................
Fruit (stewed or fresh), 1 portion.........
Water, 300 c.c.......................

8 A. M.—No food or fluid is to be given during the night or until 8 o'clock the next morning (after voiding), when the regular diet is resumed.

Patient is to empty the bladder at 8 A. M. and at the end of each period, as indicated below. The specimens are to be collected for the following periods in properly labeled bottles:

8 A. M.—10 A. M.; 10 A. M.—12 N.; 12 N.—2 P. M.; 2 P. M.—4 P. M.; 4 P. M.—6 P. M.; 6 P. M.—8 P. M.; 8 P. M.—8 A. M.

The dietary, as above given, contains approximately 13.4 gm. of nitrogen, 8.5 gm. of salt and 1760 c.c. water, and considerable purin material in the meat, soup, tea and coffee. All of these substances act as diuretics. It is on the manner of response to these stimuli that this test of renal function depends. It is not essential that all meals be eaten completely, nor exactly as indicated. In private patients it will suffice for the patient to eat three full meals, making approximate note of quantities, as one cup coffee, etc. While the above liberties may be taken, it is of special importance that the urine be collected accurately during the stated periods, that no solid or liquid food be taken between meals, and especially none at night, and that the 12 hour night specimen be completed before breakfast. The normal kidney responds so rapidly to the ingestion of fluid that these precautions are necessary.

The quantity and specific gravity of each specimen is determined. Originally, the salt and nitrogen in each specimen were estimated, but it was found sufficient to make the determination only for the total day and night specimens.

The important facts to be observed are as follows:

1. Characteristics of the day urine.
2. Quantity of water, salt and nitrogen eliminated

in 24 hours.

3. Characteristics of the night urine.

1. *Day Urine.*—The day urine shows a rhythmical response to each meal, in that the quantity of fluid increases at these periods. The diuretic response to the test meal was not found as constant as stated by Hedinger and Schlayer, and for this reason no great stress was put on it, due to its irregularity. However, a fixed or constant two hourly specimen has proved of some diagnostic moment. The specific gravity should not be fixed, but should vary inversely to the quantity.

2. *Quantity of Water, Salt and Nitrogen Eliminated in 24 Hours.*—Under normal conditions, the urinary output should be about 400 c.c. less than the fluid intake, which allows for the excretion by the skin, lungs and intestines. Nitrogen elimination should account for 90 per cent. of the nitrogen intake, the remainder passing out in the feces. Sodium chloride is entirely excreted in the urine, except in diarrhea. A marked deviation from the above figures should always cause an investigation of the patient's habits for the past few days. These may show a diet which necessitates the retention or excretion of one or more of these substances. Any marked variation suggests a more extended study. Of more importance is the concentration of salt and nitrogen. A normal kidney can easily concentrate nitrogen and salt above 1 per cent.; an abnormal kidney often cannot.

3. *Night Urine.*—Observations of the night urine are only of value when no solid or liquid food is taken during that interval. It varies within narrow limits no matter what the day specimens show. It should be small in amount, of high specific gravity and of high concentration in nitrogen.

The important points to be noted in the urine of normal individuals on the test meal for nephritic function are:

1. Variations in the specific gravity of the 2 hourly specimens, usually 9 points or more. (In case of diminished water intake and oliguria, the variations may be somewhat less.)

2. An approximate balance between the output and intake of salt, nitrogen and fluids.

3. A night urine of high specific gravity (1.016—1.018 or higher) of high nitrogen concentration (1 per cent. or more) and small in amount (400 c.c. or less) regardless of the fluid ingested or the amount of urine voided during the day.

Normal Reaction to Nephritic Test Meal.

Time of Day	Urine c.c.	Sp. Gr.	Sodium Chlorid Per Cent.	Sodium Chlorid gm.	Nitrogen Per Cent.	Nitrogen gm.
8–10	153	1.016	1.32	2.02	0.89	1.26
10–12	156	1.019	1.25	1.95	0.74	1.15
12– 2	194	1.012	0.64	1.24	0.59	1.14
2– 4	260	1.014	0.77	2.00	0.56	1.46
4– 6	114	1.020	0.99	1.13	0.95	1.08
6– 8	238	1.010	0.43	1.02	0.52	1.23
Total day....	1,115	.....	....	9.36	....	7.32
Night, 8–8...	375	1.020	0.63	2.36	1.23	4.61
Total 24 hours	1,490	.....	....	11.72	....	11.93
Intake.......	1,760	.....	....	8.5	....	13.4
Balance......	+ 270	.....	....	—3.22	....	+1.47

Impression: Normal reaction to the nephritic test meal. Note the variations occurring in the fluid output, and the specific gravity, which are in inverse ratio; the night urine, which is small in amount and shows a high specific gravity and a high percentage of nitrogen; and the approximately normal output of water, salt and nitrogen in twenty-four hours. (After Mosenthal.)

Normal Reaction to Nephritic Test Meal

Time of Day	Urine c.c.	Sp. Gr.	Sodium Chlorid Per Cent.	Sodium Chlorid gm.	Nitrogen Per Cent.	Nitrogen gm.
8–10	240	1.018				
10–12	482	1.010				
12– 2	244	1.019				
2– 4	290	1.010				
4– 6	120	1.017				
6– 8	420	1.007				
Total day....	1,796	.....	0.66	11.84	0.41	7.36
Night, 10–8..	352	1.017	0.58	2.16	1.20	4.22
Total 24 hours	2,148	.....	....	14.00	....	11.58
Intake.......	1,760	.....	....	8.50	....	13.40
Balance......	—388	.....	....	—5.50	....	+1.82

Impression: Normal reaction to the nephritic test meal. In this case, polyuria is evident. This is probably due to the reaction in an individual who is accustomed to a bland diet and not to the quantities of salt and purins taken with the test meal. (After Mosenthal.)

The Urinary Response to the Test Meal in Disease.—The normal kidney possesses the power of concentrating and diluting the urine, and readily answers the demand for either, in order to maintain the body fluids at the proper concentration. The kidney expresses its diminished power to functionate by a fixation of concentration. The loss of this flexibility has been called "hyposthenuria" by Koranyi. The result is a fixation of specific gravity. Such a fixation of specific gravity occurs in a kidney which is the seat of a nephritis. It is of great importance to remember, however, that extrarenal conditions may cause a reduction of renal function with a resulting fixation of specific gravity. Such conditions are pyelitis, cystitis associated with prostatic hypertrophy, hydronephrosis, pyo-

nephrosis, polycystic kidneys, renal congestion due to cardiac disease, diabetes insipidus, severe anaemias, and possibly other conditions. A constancy or fixation in the quantity of liquid or other constituents have not yielded anything of particular value. An impaired renal function may show a retention of one or more of the urinary constituents under observation, namely, water, salt and nitrogen. In no instance has the combination of retention of nitrogen with sufficient salt excretion been encountered.

Nycturia (an increased *amount* of urine at night, above 400 c.c.), not pollakiuria (increased frequency of urination) is to be regarded as one of the earliest signs of nephritis. It is present when the only other signs are a trace of albumin and an occasional hyaline cast. Occasionally, a nocturnal polyuria is present for which no organic lesion can be found. Possibly this may be a functional change which exhibits itself as the first symptom of kidney disease. It is always to be remembered that functional and anatomical diagnoses do not always keep pace, especially in the early stages.

Results with Complete Test Meals.—Chronic Interstitial Nephritis.—The points indicating renal insufficiency in these examples of this condition vary with the intensity of the pathological condition, and are as follows:

1. Fixed and low specific gravity.
2. Diminished output of both salt and nitrogen.
3. Tendency to total polyuria.
4. Night urine showing a slight or marked increase in volume; low specific gravity; low concentration of nitrogen.

EARLY HYPERTENSIVE NEPHRITIS

Time of Day	Urine c.c.	Sp. Gr.	Sodium Chlorid Per Cent.	Sodium Chlorid gm.	Nitrogen Per Cent.	Nitrogen gm.
8–10	465	1.009	0.51	2.37	0.34	1.58
10–12	102	1.014	0.62	0.63	0.77	0.79
12– 2	205	1.009	0.32	0.66	0.44	0.90
2– 4	160	1.010	0.28	0.44	0.64	1.02
4– 6	116	1.014	0.48	1.55	0.80	0.92
6– 8	160	1.006	0.09	0.14	0.29	0.46
Total day....	1,208	.....	....	4.79	....	5.67
Night, 8–8...	935	1.010	0.33	3.08	0.50	4.67
Total 24 hours	2,143	.....	....	7.87	....	10.34
Intake.......	1,760	.....	....	7.50	....	13.40
Balance......	—383	.....	....	—0.37	....	+3.06

Impression: The nephritic test meal shows a tendency toward fixation of specific gravity and a distinct nocturnal polyuria in an early case of hypertensive nephritis. (After Mosenthal.)

REACTION TO NEPHRITIC TEST MEAL IN ADVANCED HYPERTENSIVE NEPHRITIS

Time of Day	Urine c.c.	Sp. Gr.	Sodium Chlorid Per Cent.	Sodium Chlorid gm.	Nitrogen Per Cent.	Nitrogen gm.
8–10	133	1.010	0.36	0.48	0.35	0.47
10–12	176	1.009	0.36	0.63	0.34	0.60
12– 2	156	1.010	0.32	0.50	0.35	0.55
2– 4	212	1.009	0.36	0.76	0.34	0.72
4– 6	164	1.009	0.38	0.62	0.36	0.59
6– 8	104	1.010	0.33	0.34	0.33	0.34
Total day....	945	.....	....	3.33	....	3.27
Night, 8–8...	590	1.010	0.34	2.01	0.38	2.24
Total 24 hours	1,535	.....	....	5.34	....	5.51
Intake.......	1,510	.....	....	5.80	....	12.20
Balance......	— 25	.....	....	+0.46	....	+6.69

Impression: Reaction to the nephritic test meal in a case of advanced hypertensive nephritis. There is very marked fixation of the percentage figures for nitrogen and salt concentration and the specific gravity. There is evident nitrogen retention. The salt intake is too low to make it certain that a diminished ability to excrete salt does not exist. (After Mosenthal.)

Extreme Interstitial Nephritis

Time of Day	Urine c.c.	Sp. Gr.	Sodium Chlorid Per Cent	Sodium Chlorid gm.	Nitrogen Per Cent	Nitrogen gm.
8–10	24	1.005				
10–12	106	1.006				
12– 2	82	1.007				
2– 4	83	1.008				
4– 6	0	.....				
6– 8	230	1.008				
Total day....	525	.....	0.12	0.63	0.25	1.28
Night, 6–8...	1,140	1.007	0.12	1.37	0.20	2.27
Total 24 hours	1,665	.....	....	2.00	....	3.55
Intake.......	1,850	.....	....	6.00	....	13.00
Balance......	+ 185	.....	....	+4.00	....	+9.45

Impression: Reaction to the nephritic test meal in a case of extreme interstitial nephritis. Note the low fixed specific gravity, the retention of salt and nitrogen, and the night urine, which is increased in amount, shows a low specific gravity and a low nitrogen concentration. (After Mosenthal.)

The above results may be simulated by various disease conditions, which may be summarized as follows:

1. Causes in the urinary passages: pyelitis, cystitis, prostatic hypertrophy.

2. Causes in the blood: marked anemia.

3. Causes in the kidney (organic or functional): pyelonephritis, polycystic kidneys, diabetes insipidus.

A thorough etiologic search is therefore of great importance, especially as regards prognosis.

Renal Congestion (Myocardial Insufficiency).—The following are points characteristic of acute myocardial decomposition:

1. Specific gravity markedly fixed at the level of about 1.020.

2. A diminished output of salt. The low percentages for sodium chloride are striking.

3. An adequate nitrogen output. The very high

concentration of nitrogen is in marked contrast to that of salt.

4. An oliguria.
5. A night urine normal in character.

URINE IN EXTREME CARDIAC DECOMPENSATION

Time of Day	Urine c.c.	Sp. Gr.	Sodium Chlorid Per Cent.	Sodium Chlorid gm.	Nitrogen Per Cent.	Nitrogen gm.
8–10	61	1.018	0.20	0.12	1.52	0.93
10–12	52	1.020	0.24	0.12	1.83	0.95
12– 2	65	1.019	0.26	0.17	1.73	1.12
2– 4	55	1.018	0.27	0.15	1.65	0.90
4– 6	30	1.020	0.26	0.07	1.61	0.48
6– 8	35	1.021	0.40	0.14	1.80	0.63
Total day....	298	.....	....	0.77	....	5.01
Night, 8–8...	275	1.021	0.31	0.85	1.85	5.07
Total 24 hours	573	.....	....	1.62	....	10.08
Intake.......	570	.....	....	5.00	....	12.00
Balance......	— 3	.....	....	+3.38	....	+1.92

Impression: Nephritic test meal in an extreme case of cardiac decompensation. Note the high concentration of nitrogen as compared to the low figures for salt. There is distinct oliguria. (The water output should be higher as general anasarca was present.) (After Mosenthal.)

Hypertensive nephritis complicated by myocardial decompensation is a symptom complex met with so frequently that it demands special attention. As a rule the picture of renal congestion predominates, but occasionally it cannot "break through" the barrier imposed by the contracted kidney.

HYPERTENSIVE NEPHRITIS COMPLICATED BY MYOCARDIAL INSUFFICIENCY

Time of Day	Urine c.c.	Sp. Gr.	Sodium Chlorid Per Cent.	Sodium Chlorid gm.	Nitrogen Per Cent.	Nitrogen gm.
8–10	85	1.016	0.41	0.34	1.09	0.93
10–12	83	1.017	0.31	0.26	1.14	0.94
12– 2	100	1.017	0.41	0.41	1.04	1.04
2– 4	100	1.017	0.41	0.41	1.04	1.04
4– 6	106	1.016	0.46	0.49	0.92	0.98
6– 8	80	1.020	0.35	0.28	1.16	0.92
Total day....	554	.....	....	2.19	....	5.85
Night, 8–9...	405	1.018	0.10	0.40	1.07	4.33
Total 24 hours	959	.....	....	2.59	....	10.18
Intake.......	1,375	.....	....	7.50	....	8.80
Balance......	+ 416	.....	....	+4.91	....	—1.38

Impression: Test meal curve in a case of hypertensive nephritis complicated by myocardial insufficiency. The low salt and high nitrogen elimination are characteristic of renal congestion, as is the high fixed specific gravity. It shows how the influence of renal congestion may "break through" the functional picture presented in hypertensive nephritis. (After Mosenthal.)

During the period of elimination of edema, as might be expected from the polyuria existing at that time, the following characteristics are to be found:

1. Specific gravity low and somewhat fixed.
2. Nitrogen elimination normal.
3. Salt and water excretion exceed the amount ingested.
4. The night urine is increased in amount, has a low specific gravity, and a low percentage of nitrogen.

Cardiac Decompensation Recovery, Eliminating Edema

Time of Day	Urine c.c.	Sp. Gr.	Sodium Chlorid Per Cent.	Sodium Chlorid gm.	Nitrogen Per Cent.	Nitrogen gm.
8–10	142	1.012				
10–12	114	1.015				
12– 2	144	1.010				
2– 4	224	1.015				
4– 6	178	1.013				
6– 8	216	1.010				
Total day....	1,018	.....	0.51	4.68	0.44	4.03
Night, 8–8...	966	1.012	0.51	4.93	0.71	6.82
Total 24 hours	1,984	.....	....	9.61	....	10.85
Intake.......	1,230	.....	....	8.50	....	13.40
Balance......	— 754	.....	....	—1.11	....	+2.55

Impression: Nephritic test meal from a case of cardiac decompensation while eliminating edema fluid. Note the low and rather fixed specific gravity, the elimination of large amounts of salt and fluid and a night urine increased in quantity with a low specific gravity and a low percentage of nitrogen. (After Mosenthal.)

When all of the edematous fluid has been eliminated,

the urinary function does not immediately assume the normal type, as is usually supposed. During this period the urine passed resembles somewhat the characteristics of that of chronic interstitial nephritis, and seems to indicate that congestion has definitely impaired the function of the kidney, at least for a time. The following points are to be noted:

1. Low moderately fixed specific output.
2. Normal water and nitrogen output.
3. Slightly diminished salt output.
4. The night urine may or may not be increased, its specific gravity and nitrogen concentration is low.

FULL CARDIAC COMPENSATION FOLLOWING DECOMPOSITION

Time of Day	Urine c.c.	Sp. Gr.	Sodium Chlorid Per Cent.	Sodium Chlorid gm.	Nitrogen Per Cent.	Nitrogen gm.
8–10	198	1.005	0.27	0.54	0.27	0.53
10–12	108	1.006	0.42	0.45	0.41	0.44
12– 2	41	1.011	0.58	0.24	0.60	0.24
2– 4	95	1.009	0.54	0.51	0.59	0.43
4– 6	46	1.009	0.43	0.20	0.79	0.36
6– 8	70	1.010	0.61	0.43	0.74	0.52
Total day....	558	.....	....	2.37	....	2.52
Night, 8–8...	515	1.011	0.51	2.63	0.53	2.70
Total 24 hours	1,073	.....	....	5.00	....	5.22
Intake.......	1,360	.....	....	6.60	....	6.00
Balance......	+ 287	.....	....	+1.60	....	+0.78

Impression: Nephritic test meal from an individual with full cardiac compensation following a period of decompensation. The figures show considerable deviation from the normal: low, moderately fixed specific gravity, slightly diminished salt output, nocturnal polyuria with a low specific gravity and low concentration of nitrogen. (After Mosenthal.)

Chronic Diffuse (Parenchymatous) Nephritis.—The urinary findings in this type of nephritis are as variable as the clinical picture. During the period of edema formation the test meal shows marked salt and water retention, nocturnal polyuria and a good nitrogen elim-

ination. Such urines resemble very closely those seen in myocardial decompensation (except the nocturnal polyuria in this type), and usually require clinical data for a differentiation. This is usually easy. However, the nocturnal polyuria usually helps to indicate a parenchymatous nephritis. During the elimination of edema, the picture is similar to that of myocardial decompensation under like conditions.

CHRONIC DIFFUSE NEPHRITIS

Time of Day	Urine c.c.	Sp. Gr.	Sodium Chlorid Per Cent.	Sodium Chlorid gm.	Nitrogen Per Cent.	Nitrogen gm.
8–10	32	1.025				
10–12	0	.....				
12– 2	54	1.024				
2– 4	64	1.033				
4– 6	64	1.028				
6– 8	66	1.030				
Total day....	280	.....	0.18	0.50	1.91	5.34
Night, 8–8...	595	1.016	0.08	0.48	0.93	5.53
Total 24 hours	875	.....	....	0.98	....	10.87
Intake.......	1,760	.....	....	8.50	....	13.40
Balance....	+885	.....	....	+7.52	....	+2.53

Impression: Nephritic test meal in a case of chronic diffuse nephritis during the period of edema formation. The marked salt and water retention, the nocturnal polyuria and high nitrogen excretion are characteristic. (After Mosenthal.)

CHRONIC DIFFUSE NEPHRITIS

Time of Day	Urine c.c.	Sp. Gr.	Sodium Chlorid Per Cent.	Sodium Chlorid gm.	Nitrogen Per Cent.	Nitrogen gm.
8–10	230	1.022	1.16	2.66	1.15	2.64
10–12	130	1.025	1.08	1.40	1.42	1.85
12– 2	118	1.022	1.01	1.19	1.21	1.43
2– 4	136	1.022	1.14	1.44	1.25	1.70
4– 6	96	1.020	0.70	0.68	1.18	1.13
6– 8	108	1.014	0.80	0.87	0.89	0.96
Total day....	818	.....	....	8.24	....	9.71
Night, 8–8...	950	1.014	0.78	7.61	0.73	7.14
Total 24 hours	1,768	.....	....	15.85	....	16.85
Intake.......	1,760	.....	....	8.50	....	13.40
Balance......	— 8	.....	....	—7.35	....	—3.45

Impression: Results of the nephritis test meal in a case of chronic diffuse nephritis, while eliminating edema. Note the large amounts of fluid, salt and nitrogen excreted. (After Mosenthal.)

In Mosenthal's series it was interesting to note an occasional case showing many of the symptoms of nephritis, and a normal response to the test meal. The converse is also occasionally to be found.

Summary.—Quoting Mosenthal:

"The nephritic test meal, as suggested by Hedinger and Schlayer, and elaborated in this paper, has not only proved itself to be an admirable test for renal function, but also in many cases has been of great value in diagnosing cardiac, renal and other conditions. Much pleasure and profit may be derived from a study of diseases of the kidney from this point of view, since it forms a basis for a rational therapy, and stimulus toward keen clinical observation."

Note: The salt was estimated by the Volhardt method, the nitrogen by the Kjeldahl process.

* References: Mosenthal: Archives Int. Med., 1915, XVI, 733-774; Hedinger and Schlayer: Deutsch. Arch. f. klin. Med., 1914, cxiv, 120; Koranyi and Richter: Physiakalische Chemie und Medizin, Leipzig, 1908, ii, 136-152.

5. *Estimation of Urinary Coloring Matter as Test of Renal Function*

Thudichum's Test.—This test is now of only historical interest. It was proposed by its author on the clinical ground that in many chronic kidney diseases

the urine becomes distinctly paler in color. Therefore careful quantitative estimations of color excretion might be of value as an early sign of renal impermeability. But unfortunately for the value of the test, the quantity of coloring matter excreted in the urine depends upon a great variety of factors (liver, intestine, food, etc.), of which the most insignificant of all is perhaps renal permeability.

6. Estimation of Urinary Diastase as an Index of Renal Function

Wohlgemuth's Test.[3]—*Technic.* After neutralization urine is placed by means of an accurately graduated pipette in a series of twelve test tubes, the amount decreasing from .6 c.c., .5 c.c., .4 c.c., to .1 to .09 c.c., .08 c.c., to .04 c.c. A sufficient quantity of 1% sodium chloride solution is then added, to bring the amount of fluid in each tube up to 1 c.c. In order to more readily obtain the fractional quantity of urine required, 1 c.c. of the urine may be diluted up to 10 c.c. and from this diluted urine the required measures may be taken.

To each tube is added 2 c.c. of a 1-1000 solution of freshly prepared soluble starch. The tubes are immersed in a water bath at 38° C. for 30 minutes, after which they are placed in cold water for 3 minutes.

To each tube is then added sufficient 1/50 normal iodin solution to elicit a permanent color, violet or blue occurring where digestion is not complete.

The tube in the series immediately preceding incomplete digestion of the starch indicates the diastase content of that particular urine. From this is calculated the diastasic activity represented by δ. δ is expressed as the number of c.c. of 1/10% starch solution which

[3] Lancet Clinic, 1913, CX, p. 164.

can be digested by 1 c.c. of urine. This test can be applied to the whole urine or to the samples obtained unilaterally by ureteral catheterization. The diastase test is particularly adapted to unilateral estimations of kidney function applied to urine obtained by ureteral catheterization. But altho it is capable of indicating in the majority of cases which is the diseased or more diseased kidney, in the opinion of most genitourinary surgeons it is not necessary and adds nothing to the information obtained from the phthalein test or urea estimations, which latter are operations somewhat more easily performed.

The diastase test has never been used to any extent in estimating total functional capacity of the kidneys.

B. The Study of Physical and Biological Characteristics of the Urine as Criteria of Renal Function

There are three tests which come under this category:

1. Cryoscopy or determination of the freezing point of the urine.
2. Electrical conductivity of the urine.
3. Determination of urinary toxicity.

1. Cryoscopy of the Urine. Significance for Estimating Renal Function. Von Koranyi's Test

The theoretical bases upon which this test is founded are of extreme interest both from physical and physiological points of view but naturally cannot be completely considered here.

The freezing point of distilled water is zero. The freezing point of any solution is below zero, and the depression of freezing point below zero is proportionate to the molecular concentration of the solution. Conse-

quently the freezing point of a solution is a measure of its molecular concentration. It is also a measure of its osmotic pressure.

The freezing point of a solution is independent of the nature, size, and molecular weight of the dissolved molecules and is only dependent on their number. The specific gravity of a solution is on the contrary dependent upon the nature and molecular weight of the dissolved molecules. Solutions of similar molecular concentration have the same freezing point and the same osmotic pressure but not necessarily the same specific gravity.

By cryoscopy the molecular concentration of urine and blood can be estimated and in this way a certain insight into the functional power of the kidney can be obtained, since the global function of this organ is to regulate the osmotic pressure, or, what is the same, the molecular concentration of the blood. One can obtain from an estimation of the lowering of the freezing point of the urine below the zero of distilled water a somewhat more adequate idea of the functional capacity of the kidney than from the specific gravity.[4]

Of two urines of equal specific gravity the one with the lower freezing point comes from the better functioning kidney.

Investigations have shown that the freezing point of the urine (Δ) in health varies between rather wide limits ($\Delta =$ —.90° to —2.30°) and that it is to some extent affected by miscellaneous factors of extrarenal nature.

Altho much was expected originally from the determination of the freezing point (cryoscopy) of urine

[4] Some authors do not agree that cryoscopy is superior to sp. gr. estimation in determining renal permeability. v. Sahli, Diagnostic Methods, 1905, p. 551.

in estimating the integrity of renal function, it is not depended upon to any great extent at present.

The molecular concentration of the blood hence its freezing point (δ) is much more constant than that of the urine. It is supposed to remain somewhere near —.56°. It was thought that insufficiency of renal function by allowing the accumulation of molecules in the blood which should be excreted would raise the molecular concentration therein or, in other words, lower the freezing point. Unfortunately, experience has not confirmed the hopes of those who thought that cryoscopic examinations of the urine and blood would solve the great problem of estimating the renal functions and the method is not extensively used.

The relation between the lowering of freezing point of blood (δ) and urine ($\triangle$) was proposed by Dreser as a measure of the work done by the kidney and by Bernard as the basis of a sort of mathematical conception of the eliminatory power of the kidney. The formula $\frac{\delta}{\triangle} \times V = R$ will represent the molecular elimination of the kidney according to this conception. δ represents the freezing point of urine, $\triangle$ that of the blood, V the quantity of urine in 24 hours. In normal cases R varies from 3000 to 5000, whereas in renal insufficiency these numbers are considerably reduced. Unfortunately all attempts to reduce our conceptions of organic function to mathematical terms have not been eminently successful.

Technic of Cryoscopy.—The technic of cryoscopy is not especially complex but requires a certain apparatus for its performance. It is usually carried out in the laboratory and has never become a routine clinical procedure. Only the necessary outlines of the method need here be given since those who desire

to master it can easily refer to numerous texts in which the technique is minutely described.[5]

The technic of cryoscopy is carried out with Beckmann's freezing apparatus carrying a special thermometer. The freezing mixture is made of ice, water and salt. In the freezing mixture is plunged an ordinary thermometer and a mixer, thus enabling the temperature to be kept at a fixed point (—3° to —5°). Through an opening a tube containing the special thermometer immersed in the liquid to be frozen can be immersed in the freezing mixture. The estimation of the exact points of freezing is not difficult and usually the operation can be performed in its entirety in 15 or 20 minutes.

2. *Electrical Conductivity of the Urine*

The electrical conductivity of the urine in health and disease was first studied by Turner. The electrical conductivity is estimated in ohms of resistance and depends upon the number of ions of salts dissolved in the urine. The method measures, in other words, the amount of salts or mineral content of the fluid. The Kohlrausch method of performing the test, which is the one usually employed, requires a whetstone bridge, a resistance box, telephone and cells, besides other paraphernalia, and partly on account of this complexity and also the fact that the practical results obtained are meagre, the test has never come into general use.

[5] Consult in this connection Wood's Chemical and Microscopical Diagnosis, 1909, p. 61; Sahli's Diagnostic Methods, p. 546.

3. Estimation of Urinary Toxicity as a Test of Renal Function

Bouchard's Test.—It was for a long time believed that the toxicity of the urine was proportional to the functional power of the kidney. Urine of human beings produces symptoms of poisoning and death when injected intravenously into lower animals. Bouchard developed from this fact a method of testing renal function [6] by determining the quantity of a 24-hour specimen of urine, required to kill a kilogram of lower animal. Bouchard established a so-called urotoxic coefficient which was that quantity of poison elaborated by every kilogram of body weight of the person whose urine was tested.

The same objections exist with respect to the theoretical basis of this test, as in the case of chemical urinalysis previously discussed, namely that so many factors besides renal sufficiency or insufficiency enter into the production of results that the test becomes devoid of scientific value. It simply shows the toxicity of a given urine injected intravenously into a given animal and by no means reflects the actual functional power of the kidney through which it was derived. The test is quite complex and has been abandoned.

II. STUDIES OF THE BLOOD AS CRITERIA OF RENAL FUNCTION. ESTIMATION OF BLOOD UREA AND OF INCOAGULABLE (RESIDUAL) NITROGEN IN BLOOD

When it is considered that a major portion of the nitrogenous waste of the body makes its escape through the kidney by way of the urine, it becomes evident that a diminution of the functional capacity of these organs

[6] Also toxopexic liver function (*v. s.*).

must often result in an accumulation of nitrogenous products of metabolism in the blood.

Such an idea is very old and as long ago as 1821 Prevost and Dumas [7] reported an increase of urea in the blood after extirpation of the kidneys in animals. The clinical importance of their experiments was recognized by Bright in his observations upon nephritis in 1836.[8]

After Bright's time it became well recognized that in chronic nephritis there may be a tendency toward accumulation of nitrogenous matters in the blood, but the quantitative study of nitrogen retention was forced to await the development of accurate chemical methods of investigation. In this place we can speak only of modern technic.

The development of accurate technic in nitrogen estimation of the blood and its application to clinical medicine is a subject which has been perfected only since the beginning of the century. Ascoli,[9] Strauss [10] and others showed that in many cases of chronic Bright's disease nitrogenous matter accumulates in the blood and the increase is more marked as death approaches. Muller [11] showed that in outspoken uremia the accumulation becomes proportionately more marked.

Obermayer and Popper [12] first laid stress upon the increase of incoagulable nitrogen in the blood serum in uremic states and found that in this incoagulable nitrogen increase, urea plays the most important part.

[7] Quoted by Schondorff in Pflüger's Archiv f. de ges. Physiol., 1899, LXXIV, p. 307.

[8] Guy's Hosp. Rep., 1836, I, p. 358.

[9] Pflüger's Arch. f. de ges. Physiol., 1901, LXXXVII, p. 103.

[10] Chronisch Nierenenzundung; ihrer Einwirkung auf die Blutflussigkeit, etc., Berlin, 1902.

[11] Verh. d. Deutsch. path. Gesell., 1904-5, VII to IX, Erg. 80.

[12] Zeit. f. klin. Med., 1909, LXVII, p. 332.

Hohlweg[13] substantiated these facts and altho he showed that an increase of urea in the blood is not necessarily pathognomonic of uremia, nevertheless its accumulation therein may be regarded as an evidence and to some extent at least as an index of the function of the kidneys.

Widal,[14] however, believes that the amount of urea retention is an actual quantitative index of renal function and that the severity and prognosis of a given case may be predicated upon the basis of such findings. Widal's clinical method of estimating urea in the blood is not commonly used in this country as it gives, according to Rowntree and Fitz, an error of 10 to 60% and is therefore useless as a quantitative method.

The estimation of urea in blood serum has remained until recently a rather difficult chemical operation, but of late one or two practical clinical methods have been devised which render the test much more practical. Marshall's method appears to be the simplest and most practical of these and will be described in full (*v.i.*).

The normal figures for the elimination of urea are between .300 to .500 gm. per liter of serum.

The simplest methods by which the total incoagulable nitrogen in the blood serum may be determined still remain even more difficult and complicated than those of urea estimation, especially since they involve in the end a nitrogen determination by Kjeldahl's method. A fair laboratory equipment is therefore necessary.

The most practical method of determining the incoagulable nitrogen seems to be that of Hohlweg and Meyer which has been modified by Morris[15] in

[13] Deut. Archiv f. klin. Med., 1912, CIV, p. 216.

[14] Bull. et Mem. Soc. Méd. d. hôp. de Paris, 3, 1911, XXXII, p. 627.

[15] Archiv. of Int. Med., 1911, VIII, p. 457.

this country. This method will be described below. The normal figures for total incoagulable nitrogen in the blood serum are .500 to .600 gm. per liter.

The recent work of Folin and Denis indicates that a urea concentration in the blood of .5 gm. and total incoagulable nitrogen content of .6 gm. per liter, which was formerly considered normal, is too high an estimate. They found the normal urea concentration .13 gm. and incoagulable nitrogen .26 gm. per liter. In their experience no great prognostic significance is to be attached to urea concentration of less than .55 gm. per liter and incoagulable nitrogen less than .50 gm. per liter. Greater concentration than this, especially if the freezing point of the serum drops lower than —60 are of considerable prognostic significance.

In pure passive congestion Rowntree states that he has never seen the rest nitrogen in the blood serum higher than .63 gm. per liter.

Marked accumulation of incoagulable nitrogen or of urea in the blood is now regarded as a valuable evidence of renal insufficiency and in cases of nephritis it is an unfavorable prognostic sign.

The relation between non-protein (incoagulable) nitrogen retention in the blood and the excretion of phenolsulphonphthalein has been studied in experimental uranium nephritis by Frothingham, Fitz, Folin and Denis.[16] These investigators found that the results of the two tests paralleled very closely. For this reason and because in clinical studies the same parallelism has been found to obtain, these two tests have come to be considered as among the best for conjoint use.

It will now be necessary to give the simplest, most practical and accurate methods by which the clinical investigator may determine the two important phases

[16] Arch. of Int. Med., 1913, XIII, p. 245.

of the blood which have been discussed, namely, the amount of urea in the blood and the amount of incoagulable or rest nitrogen contained in the same fluid. The chemical operations which are used at present for these purposes are simple enough to bring about their frequent use in the clinic. The best method of quickly and accurately determining the amount of urea in the blood is that of Marshall. The incoagulable or rest nitrogen in blood serum is usually determined by either of two general methods, that of Hohlweg-Meyer and that of Folin and Denis. The details of these three important methods will now be given.

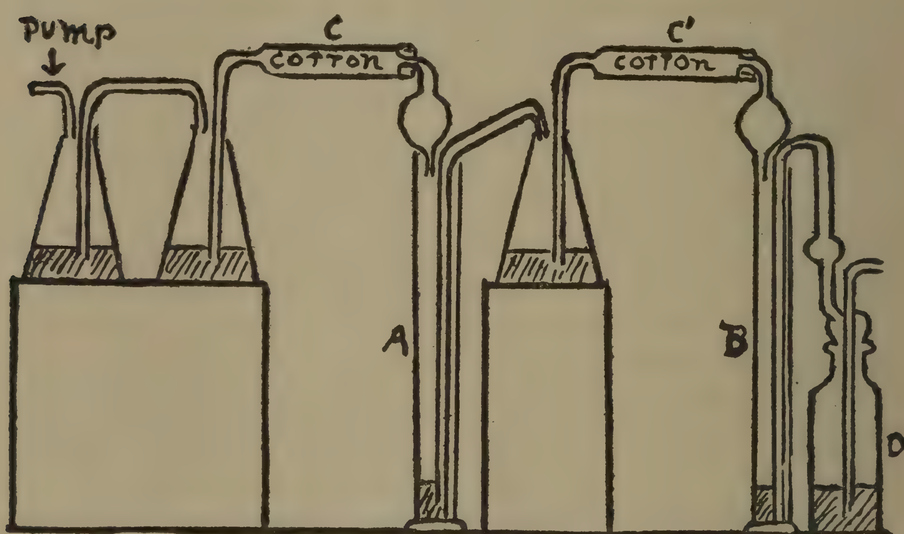
Marshall's Method for the Determination of Urea in the Blood.[17]—The blood is drawn in the usual manner and allowed to stand on ice until clotting is complete. As shown below, the urea content of the serum does not change after standing even for three or four days; the blood can, therefore, be kept on ice over night, if desired.

Two equal portions of the serum are measured into ordinary test tubes, 1 c.c. of the soy bean extract[18] added to one tube, and about 0.5-1.0 c.c. of toluene to each. If sufficient serum is available, 10 c.c. portions should be used; however, perfectly satisfactory results can be obtained by using 5 c.c. or even 3 c.c. portions of the serum. The tubes are tightly stoppered and allowed to remain at room temperature until the conversion of the urea into ammonium carbonate is complete. Generally, they are allowed to stand over night,

[17] Jour. of Biol. Chem., 1913, XV, No. 3.

[18] The preparation of the soy bean extract is as follows: Ten grams of finely ground soy beans are treated with 100 c.c. of water and allowed to stand with occasional agitation for one hour. 10 c.c. of $\frac{n}{10}$ hydrochloric acid are added and the mixture allowed to stand about fifteen minutes longer. It is now filtered and preserved with toluene. Such a solution is perfectly satisfactory for use at least five or six days after its preparation.

altho four to five hours is usually amply sufficient for the completion of the reaction. The contents of the tube containing the serum and extract are transferred to cylinder A (see illustration), and washed in with a very small amount of water (not more than 5 c.c.). Two grams of sodium chloride, an equal volume of alcohol and a layer of kerosene oil are added to the cylinder. The contents of the other tube are transferred to



cylinder B, and treated in exactly the same manner. 25 c.c. of $\frac{n}{50}$ hydrochloric acid and about 25 c.c. of water are placed in each of the 200 c.c. Erlenmeyer flasks used for the absorption of the ammonia. The different parts of the apparatus are now connected and about 0.5 gram of sodium carbonate added to each cylinder. A rapid air current is passed through the apparatus until all the ammonia has been removed from the cylinders. With a good suction pump, one hour suffices. The

excess of acid in the absorption flasks is titrated with $\frac{n}{50}$ sodium hydroxide and alizarin sodium sulphonate. The amount of acid neutralized in the flask attached to cylinder B corresponds, of course, to the ammonia[19] present in the serum, while the amount used in the other two flasks represents the urea plus the ammonia. The difference corresponds to the urea in terms of $\frac{n}{50}$ hydrochloric acid, and multiplied by 0.0006 gives the urea in grams present in the amount of serum taken for the determination.

Details in Connection With the Apparatus and Determination.—1. On account of the large quantity of protein in serum, it is advisable to use both alcohol and kerosene to prevent foaming.[20]

2. The tubes C and C′ are ordinary calcium chloride drying tubes packed loosely with cotton. These in conjunction with the bulbs prevent any splashing or mechanical transmission of the alkali into the absorption flasks. While the bulbs are probably not absolutely necessary, they are convenient in keeping the cotton filters dry.

3. For the better absorption of the ammonia, the tubes in the Erlenmeyer flasks are closed at one end, and pierced with six or seven small holes, as suggested by Folin.[21] Even with this device one absorption flask is not always sufficient to completely absorb the ammonia. Two flasks are always used for safety in con-

[19] We can, however, place no value on this as a determination of the true ammonia content of the blood, for on standing even a few hours the blood develops much more ammonia than the original amount (Folin).

[20] This has been pointed out by Folin, in connection with the use of the air current method for determining ammonia in blood. (Zeitsch. f. physiol. Chem., XXXVII, p. 165, 1902-03.)

[21] Jour. Biol. Chem., XI, p. 493, 1912.

nection with the urea determination; however, since from the serum alone only a very small amount of ammonia (corresponding to 0.10-0.70 c.c. of $\frac{n}{50}$ HCl) is ordinarily obtained, one absorption flask is here sufficient.

4. A layer of toluene is placed on the liquid in the absorption flasks, for, due probably to the alcohol carried over by the air current, considerable foaming sometimes occurs. If not prevented, this results in a loss of a portion of the contents of the flask.

5. The bottle contains dilute sulphuric acid to free the air from any traces of ammonia before passing it through the apparatus.

6. No correction is necessary for the ammonia derived from the 1 c.c. of soy bean extract used, as the amount obtained from this source is unappreciable.

Another method also suggested by Dr. Marshall is to draw 5 c.c. of blood from a vein with a hypodermic needle, into a 5 c.c. pipette, and immediately transfer the specimen to a test tube, containing 1 to 2 c.c. of 1% sodium oxalate solution. To this is added 25 milligrams (one tablet) of Urease-Dunning, the tablet having been previously crushed and dissolved in 5 c.c. of water. This mixture is allowed to stand until the urea of the blood is decomposed; at ordinary room temperature, one-half an hour is usually sufficient; it is better, however, to place the test tube in a beaker of water at 30° to 40° C. for one-half hour. After the urea has been changed, the contents and sufficient washings of the tube are transferred to a cylinder. The ammonia is then removed by a current of air collected in the $\frac{n}{50}$ hydrochloric acid and titrated with $\frac{n}{50}$ sodium hydroxide.

Calculating Urea Content.—As the purpose in using Urease-Dunning is to convert the urea present in a specimen into an easily estimated substance—ammonium carbonate—and as the amount of this salt produced from this source, by the enzyme, is indicated by the increased alkalinity of the specimen to methyl orange, it is obvious that the quantity of standard hydrochloric acid required to exactly neutralize the contents of the flask containing urease, less the quantity required for the control specimen, corresponds to the ammonium carbonate formed by the conversion of the urea originally present in the specimen.

By the following equation:

$$CO\begin{matrix} NH_2 \\ \\ NH_2 \end{matrix} + 2H_2O = CO\begin{matrix} ONH_4 \\ \\ ONH_4 \end{matrix}$$

it may be calculated that 60 grams of urea would be converted, by urease, into 96 grams of ammonium carbonate, which amount would require 72 grams of hydrochloric acid to neutralize it.

As this quantity (72 grams) of hydrochloric acid is contained in 20,000 c.c. of decinormal (N/10) hydrochloric acid solution and is equivalent to 60 grams of urea, as represented by 96 grams of ammonium carbonate, it follows that one twenty-thousandth of this quantity or 1 c.c. of decinormal hydrochloric acid would be equivalent to one twenty-thousandth of 60 grams = 3 milligrams ($60 \div 20{,}000 = .003$), therefore each c.c. of decinormal hydrochloric acid required to neutralize an enzyme-treated specimen, that is in excess of the number of cubic centimeters required to neutralize the control specimen, represents three milligrams of urea, and, as the 5 c.c. specimen is the one two-hundredth part of a liter, it will be only necessary

to multiply the number of c.c. of the decinormal hydrochloric acid solution, in excess of the control's requirements, by the factor .6 ($.003 \times 200 = .6$) to ascertain the urea per liter, when estimating the daily output.

1. Technic of Estimating Total Incoagulable or So-called Rest or Residual Nitrogen in the Blood Serum

Two methods are in common use. These are Morris' modification of the Hohlweg-Meyer method, and the method of Folin and Denis.

Morris' Modification of Hohlweg-Meyer Method.—To 10 c.c. of blood serum obtained by venipuncture or otherwise, in a 300 c.c. Erlenmeyer flask is added a reagent consisting of equal parts of 1% acetic acid and a 5% solution of monocalcium phosphate, until the reaction is acid to litmus but neutral to Congo red. The volume is brought up with distilled water to 80 c.c. and 80 c.c. of saturated solution of sodium chloride are poured into the flask.

The mixture is boiled to precipitate the coagulable proteins, and the filtrate, from which the proteins have been shown to be completely removed, subjected to a nitrogen determination by Kjeldahl's method.

For a description of the technic of Kjeldahl's method see page 32.

Folin and Denis Method.—This method is considered at the present time as the most practical way of quantitatively determining the amount of rest nitrogen in the blood. It will be given in the words of its authors from their communication published in 1912. (Jour. Biol. Chem. 1912, XI, 527, Ibid. 1913, XIV, 29.)

Method for Drawing Blood.—"Before going into details of the chemical work it would seem worth while to describe our method of drawing blood because so

far as we have been able to learn it is somewhat different from the procedures employed by physiologists and because we believe it to be expeditious, neat and exact and therefore particularly suitable for quantitative work.

"We use neither cannulæ nor syringes but simply hypodermic needles and pipettes. The needles are about 1 mm. in diameter, and about 25 mm. long. They are immersed in a dilute solution of vaseline in ether and then allowed to drain and dry on a clean paper for a few minutes before being used. (This does not apply of course to the drawing of human blood when the needles must be thoroughly sterilized.) An adequate supply of these needles is kept on hand so that we do not need to use any needle more than once in any one experiment. The needle is attached to the tip of a 2 or 5 c.c. pipette by means of a short piece of narrow pure gum tubing. A small pinch of powdered potassium oxalate is introduced into the upper end of the pipette (which must be clean and perfectly dry) and is allowed to run down into the tip and the needle. The other end of the pipette is connected with a rubber tube which in turn connects with a mouthpiece consisting of a short tapering glass tube. Close to the pipette the rubber tube carries a pinchcock.

"To draw the blood insert the needle in the vein or artery and regulate the flow of the blood by means of the pinchcock and by suction. The exact quantity of blood desired is thus obtained without any waste and without clotting."

Isolation of Non-Protein Nitrogenous Constituents.—"To separate the non-protein nitrogenous constituents from the protein materials we make use of pure (acetone-free) methyl alcohol and an alcoholic solution of zinc chloride. Ordinary methyl alcohol cannot

be used because the impurities in it, particularly the acetone, combine with more or less of the urea so that it escapes decomposition in the subsequent treatment and is not quantitatively recovered. We have satisfied ourselves by means of determinations on pure urea solutions that the presence of acetone results in a loss of urea.

"As soon as the blood is drawn it is transferred into measuring flasks half filled with methyl alcohol and the flasks are then filled up to the mark with methyl alcohol and vigorously shaken. Two cubic centimeters of blood are diluted to 25, while for 5 c.c. of blood use 50 c.c. flasks. At the end of two hours, or as soon after that as is convenient, the contents of the flasks are filtered through dry filters. To the filtrate are then added two or three drops of a saturated alcoholic solution of zinc chloride and after standing for a few minutes the mixture is again filtered through a dry paper. The zinc chloride brings down an appreciable precipitate and the last traces of coloring matters so that when the second filtration is made, a perfectly colorless filtrate is obtained. 5 c.c. of these filtrates, corresponding to 0.4 or to 0.5 c.c. of blood, depending on whether 2 or 5 c.c. of blood were drawn, are taken for each determination.

"The precipitation procedure described above is the one which we ordinarily use. There are objections to it. We are not certain that protein-like materials may not escape precipitation by this as by every other method and we do know that the filtrate does not contain all of the non-protein materials. When relatively large quantities (equivalent to 100 mgm. of nitrogen per 100 c.c. of blood) of creatine or asparagine are added to blood and treated as described above there is invariably an appreciable loss of material. To over-

come this loss we have tried to triturate and wash the first alcoholic precipitate with methyl alcohol, and with some substance as, for example, with glycocoll, urea and acetamide, we are thus able to get practically quantitative results, while with others, such as creatine, asparagine and tyrosine, we still do not get quite all. Moreover, such trituration and washing does leach out a small amount of the coloring matters of the blood so that except for special experiments with less soluble substances we consider the simpler procedure rather more satisfactory.

Determination of the Total Non-Protein Nitrogen.—"To determine the non-protein nitrogen of the blood 5 c.c. of the alcoholic filtrate is transferred to a large Jena test tube. One drop of sulphuric acid, one of kerosene and a pebble are added and the methyl alcohol is driven off by immersing the test tube in a beaker of boiling water for five to ten minutes. When the alcohol is removed 1 c.c. of concentrated sulphuric acid, a gram of potassium sulphate, and a drop of copper sulphate solution are added and the mixture is boiled, cooled and diluted.

"From this digestion mixture the ammonia is removed in the usual manner. It is, however, not collected directly in a measuring flask (as in urine analysis) but in a second large test tube previously charged with 1 c.c. of $\frac{n}{10}$ acid added to 3 c.c. of water. The reason for this variation is that 0.4 to 0.5 c.c. of blood contains only 0.1 to 0.2 mgm. of non-protein nitrogen. The final Nesslerized solution cannot be diluted to 100 c.c. and smaller volumetric flasks cannot be used as receivers during the air current treatment because of spattering. Large test tubes are therefore used as receivers and the ammonia is Nesslerized in these before

the liquids are transferred to measuring flasks.

"Ordinarily the colored solutions when obtained from cat's blood are transferred to 25 c.c. flasks and are then found to have a depth of color which permits of a sure and accurate reading in the colorimeter. In some of our absorption experiments the total non-protein nitrogen runs up to very high figures and then the solutions are diluted to 50, sometimes even to 100 c.c., before being read in the colorimeter.

"Human blood contains scarcely more than one-half as much non-protein nitrogen as cat's blood. In the case of human blood we therefore never draw less than 5 c.c. and we take 10 c.c. of the filtrate for each determination. In all other respects we use the same procedure for human blood as for cat's blood.

"In all ordinary cases 7 to 8 c.c. of diluted Nessler's reagent (dilution 1:5) are added for the production of the color. If much ammonia is present so that the resulting colored solution must be diluted to 50 or 100 c.c. correspondingly larger amounts of Nessler's reagent are added.

"The calculation of the analytical results to milligrams of nitrogen per 100 c.c. of blood is not difficult, but the formulæ given below may prove useful. In these formulæ, the standard solution contains 1 mgm. of nitrogen (as ammonium sulphate) Nesslerized in a 100 c.c. flask and the colorimeter prism of the standard is set at 20 millimeters. $\frac{50}{R} \times D$, in which R stands for the reading of the unknown and D represents the volume to which its ammonia has been diluted, gives the desired figure. The reason for the figures is that we are working with 4 c.c. of blood.

"When 5 c.c. of blood is taken and it is diluted to 50,

the formula becomes $\frac{40}{R} \times D$.

"When working with human blood and taking 10 c.c. of the filtrate obtained from 5 c.c. of blood diluted to 50 the formula is $\frac{20}{R} \times D$.

"It may be thought that we are using unnecessarily small amounts of blood in these analyses. We are, however, by no means sure that working with larger amounts would yield more accurate results and we have satisfied ourselves by scores of duplicate analyses that the method as outlined gives trustworthy figures. Further, the smaller the quantity of blood which can be made to give reliable results the greater becomes the usefulness of the method. The work which we have already done on cats could not have been done on such a small animal except by means of these micro-chemical methods. Finally, small amounts of blood must be used for the urea determinations because of the disturbing effects of the sugar present."

2. *Ambard's Coefficient of Urea Excretion and the Index of Urea Excretion (McLean)*

It has been proven that the excretory activity of the kidney cannot be measured by estimations of the blood urea or rest nitrogen alone. The concentration of urea in the blood varies within wide limits. The line of demarcation between the normal and abnormal individual is not sharp, as very frequently an individual with impaired urea function will show a blood urea within the upper normal limits. Therefore, only the more marked instances of impaired renal function will be demonstrated by blood urea estimations. Even the blood urea of nephritic subjects is not fixed or con-

stant. It has been demonstrated that the concentration of the urea in the blood may be varied at will by the diet. Appreciating these variations, Ambard began a study of urea excretion in which the concentration of the urea was determined in both the blood and urine. He also added determinations of the rapidity with which urea elimination occurred. All these factors, which are interdependent, are included in his estimations. He formulated what are now known as Ambard's laws, and demonstrated a constant ratio between the concentration of urea in the blood and the rate of excretion. This numerical constant is known as Ambard's coefficient, and is usually indicated by the symbol "k." When applied to normal individuals, remarkably constant values are obtained with the coefficient. In pathologic subjects, there is an increase in the concentration of the urea in the blood and a relative decrease in the rate of excretion. Ambard has shown these relative changes to produce a marked change in the urea coefficient. Ambard's work has been repeatedly confirmed.

The principles underlying Ambard's[a] laws and coefficient, and McLean's modification are expressed very clearly and concisely by Smith in a recent publication.[b] He is quoted below.

Ambard found that:

1. When the concentration of the urea in the urine is constant, the rate of excretion varies directly as the

[a] Ambard, L.: Rapports entre le taux de l'urée dans le sang et l'élimination de l'urée dans l'urine, Compt. rend. Soc. de biol., Nov. 19, 1910, p. 411; Rapports de la quantité et du taux de l'urée dans l'urine, la concentration de l'urée du sang étant constant, ibid., Dec. 3, 1910, p. 506; Physiologie normale et pathologie des reins, Paris, 1914.

[b] Smith, R. L. I., Renal Function in Nephritis, Jour. A. M. A., 1917, lxviii, 278.

square of the concentration of urea in the blood. This law is expressed:

$$\frac{(\text{Urea in blood})^2}{\text{Rate of excretion}} = \text{Constant}$$

2. When the concentration of urea in the blood is the same, the rate of excretion varies inversely as the square root of the concentration in the urine.

$$\frac{\text{Rate of excretion I}}{\text{Rate of excretion II}} = \frac{\sqrt{\text{Concentration II}}}{\sqrt{\text{Concentration I}}}$$

$$\text{or, Rate I } \sqrt{\text{Concentration I}} = \text{Rate II } \sqrt{\text{Concentration II}}$$

Introducing this factor into the first law we have:

$$\frac{\text{Urea in blood}}{\sqrt{\text{Rate of excretion } \sqrt{\text{Concentration in urine}}}} = \text{Constant}$$

3. Other factors remaining constant, the rate of excretion varies directly with the weight of the individual.

$$\frac{\text{Rate}}{\text{Weight}} = \text{Constant}$$

Combining this with the previous formula, we have:

$$\frac{\text{Concentration of urea in blood}}{\sqrt{\frac{\text{Rate}}{\text{Weight}} \sqrt{\text{Concentration in urine}}}} = \text{Constant}$$

This formula expresses all of Ambard's laws in the simplest form. In order to standardize the formula for use in human subjects, Ambard expresses it as follows:

$$\frac{\text{Ur}}{\sqrt{\text{D} \times \frac{70}{\text{Wt}} \sqrt{\frac{\text{C}}{25}}}} = \text{Constant (k)}$$

Ur = Grams of urea per liter of blood.
D = Grams of urea excreted per twenty-four hours.
Wt = Weight of individual in kilograms.
C = Grams or urea per liter of urine.

[2] See note c, page 133.

This formula is known as Ambard's coefficient, and the value of k usually found in the normal human subject is 0.08 (McLean). The introduction of a standard weight of 70 kg., a standard concentration of 25 gm. per liter of urine and the expression of excre-

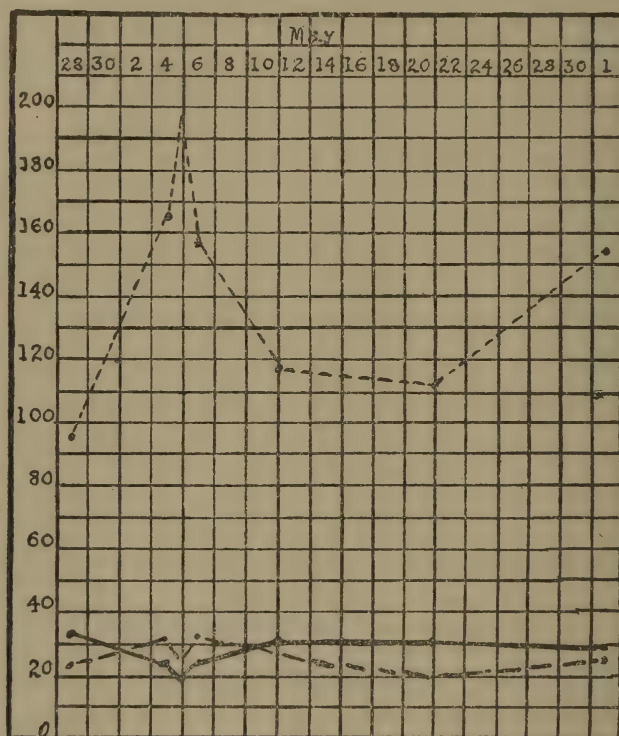


Chart 1.—Normal: solid line, milligrams of urea per hundred c.c. of blood; broken line, grams of urea excreted per twenty-four hours; dotted line, index of urea excretion. This chart shows the variations as found by McLean in a healthy adult.

tion as the rate per twenty-four hours are purely arbitrary, and do not affect the general relationship between the variable factors. When these arbitrary factors are kept constant, they, in conjunction with the constant relationship between the four variable factors, tend to keep k constant, and the actual numerical

value of k is determined by these arbitrary additions to the formula:

Index of Urea Excretion.—To obviate the necessity of an arbitrary scale for the interpretation of Ambard's coefficient, McLean[c] has adopted a formula from Ambard's laws, based on a scale of 100 so that the rate of urea excretion is given in per cent. of the normal, an index of 100 corresponding to a value of 0.08 for Ambard's coefficient.

The derivation of the index is as follows:

$$\text{Index} = \frac{\text{Rate of excretion}}{\text{Standard normal rate}} \times 100$$

From Ambard's laws:

$$1.\quad K = \frac{\text{Ur}}{\sqrt{\text{Rate}}} \qquad \therefore\ \text{Rate} = \left(\frac{\text{Ur}}{0.08}\right)^{c}$$

$$2.\quad 0.08 = \frac{\text{Ur}}{\sqrt{\text{Normal rate}}} \qquad \therefore\ \text{Normal rate} = \left(\frac{\text{Ur}}{0.08}\right)^{c}$$

$$3.\quad \frac{\text{Rate}}{\text{Normal rate}} = \frac{\left(\frac{\text{Ur}}{\text{k}}\right)^2}{\left(\frac{\text{Ur}}{0.08}\right)^2} = \left(\frac{0.08}{\text{k}}\right)^2$$

$$4.\quad \text{Index} = 100 \times \left(\frac{0.08}{\text{k}}\right)^2 = \left(\frac{0.08}{\text{k}}\right)^2$$

Substituting for k (Ambard's coefficient) and simplifying,

$$\text{Index} = \frac{\text{Gm. urea per 24 hrs.} \quad \sqrt{\text{Gm. urea per liter urine} \times 8.96}}{\text{Wt. in kg.} \times (\text{Gm. urea per liter of blood})^2}$$

When k = 0.08 the standard normal. Index = 100.

c McLean and Selling: Urea and Total Non-Protein Nitrogen in Normal Human Blood: Relation of Their Concentration to Rate of Elimination, Jour. Biol. Chem., 1914, 19, 31. McLean, F. C.: The Numerical Laws Governing the Rate of Excretion of Urea and Chlorides in Man, Jour. Exper. Med., 1915, 22, 212, 366; Clinical Determination of Renal Function by an Index of Urea Excretion, *The Journal* A. M. A., Feb. 5, 1916, p. 415.

Methods.—Specimens: The methods recommended by McLean have been used with slight modification. The observations may be made at any time, since the application of the laws is independent of the nitrogen intake. However, it has been considered preferable as a routine to make the observations in the forenoon, no food or water being taken during the period.

The patient is given 300 c.c. of water to drink. One half hour later the bladder is emptied, by catheter if necessary, and the time noted. Then after one or two hours the bladder is again emptied, the time carefully noted, and the amount of urine measured to within 1 c.c. The shorter period is used when the patient is secreting a fairly large amount of urine. At about the middle of the period, 10 c.c. of blood are drawn from a vein into a dry tube containing about 1 grain of potassium oxalate, with which it is mixed to prevent clotting.

Analysis: The urease method introduced by Marshall[d] has been used. This depends on the breaking down of urea into ammonia and carbon dioxid by the specific enzyme found in soy bean and the subsequent determination of the ammonia. The permanent preparation of urease described by Van Slyke and Cullen[e] has been used throughout. Three analyses were made in each case: blood urea, urine urea and urine ammonia. The urine ammonia is determined in order to correct the urea figure for preformed ammonia, which is also determined in the urea method.

d Marshall, E. K., Jr.: A Rapid Clinical Method for the Estimation of Urea in Urine, Jour. Biol. Chem., 1913, xiv, 283; A New Method for the Determination of Urea in Blood, ibid., 1913, xv, 487; The Determination of Urea in Urine, ibid., 1913, xv, 495.

e Van Slyke, D. D., and Cullen, G. E.: A Permanent Preparation of Urease and Its Use in the Determination of Urea, Jour. Biol. Chem., 1914, xix, 211; id., *The Journal* A. M. A., May 16, 1914, p. 1558.

Calculation of the Index: From the results of the determinations the following calculations are made:

1. Grams of urea excreted per 24 hours = D.
2. Grams of urea per liter of urine = C.
3. Grams of urea per liter of blood = Ur.

These values in the body weight in kilos are substituted in the formula for the index as given above, and the value of the index calculated by aid of a slide rule. With the modified slide rule described by McLean it may easily be calculated in fifteen seconds.

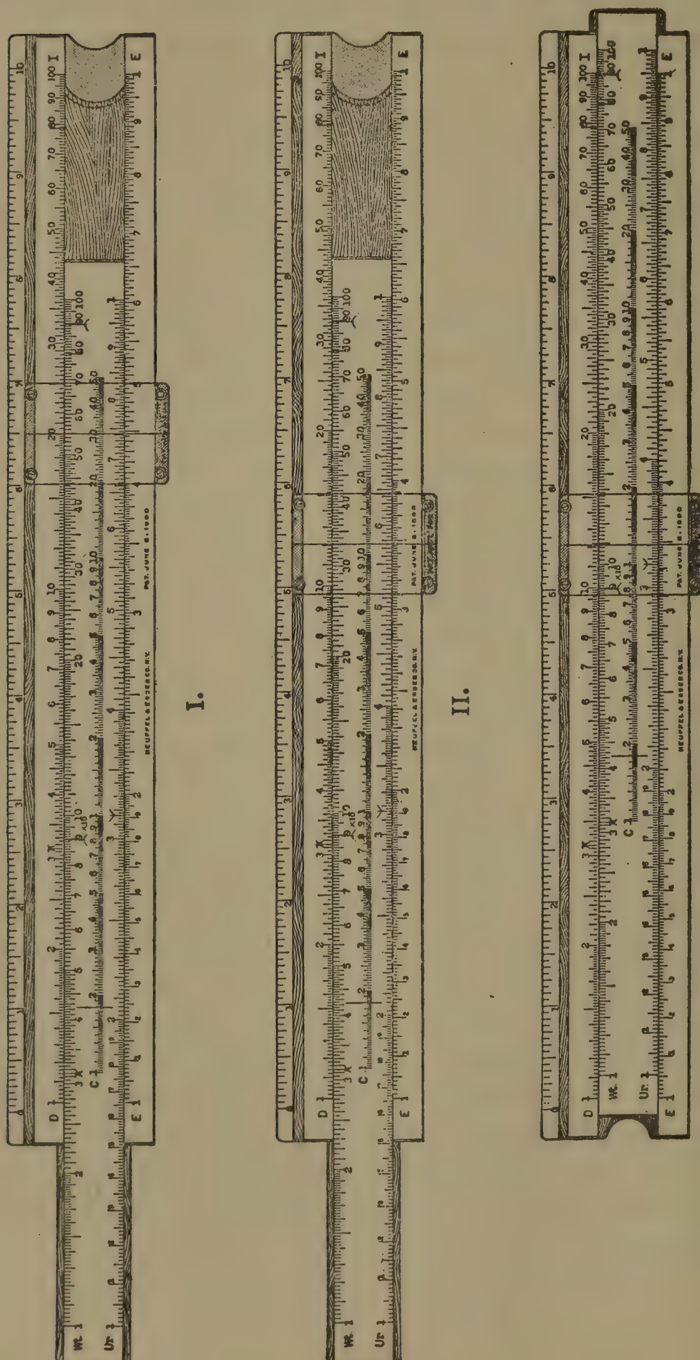
Nitrogen Intake.—It has been clearly demonstrated that while the nitrogen intake has a direct and marked influence on the concentration of blood urea, it has no effect on the index of urea excretion.

The character of the diet in this series has been included in the tables after each observation. The diet noted as "nephritic" is one which is low in protein and sodium chlorid.

Many workers, including McLean, prefer a 72-minute period, as it is one-twentieth of 24 hours, making the mathematics more simple, and is long enough to insure accuracy. Even a 36-minute period may suffice, but it is a little too short for accurate work.

While the application of this with other functional methods will be discussed in the summary, it might be well to state here that many cases of kidney involvement, especially chronic nephritis, with hypertension, show a blood urea without normal bounds, and yet show a decided impairment of renal function by the index of urea excretion.

UREA INDEX SLIDE RULE



I. II. III.

Note: The slide rule modified for the calculation of the urea index by McLean can be purchased from the Keuffel and Esser Co., New York, who will also send complete directions for its use.

3. *Creatinin and Uric Acid Estimations in the Blood*

According to Chase and Meyers, although considerable attention has been given to the urea and non-protein nitrogen of the blood, in nephritis, scant consideration has been accorded uric acid and creatinin. Despite the fact that most of the nitrogen is eliminated in the form of urea, it does not necessarily follow that studies concerning the fate of uric acid and creatinin are unimportant and uninteresting. They do not believe that it is possible to draw satisfactory deductions regarding nitrogen metabolism from the urea and non-protein nitrogen determinations alone. Both Mosenthal and Foster have referred to the fact that the increase in the non-protein nitrogen in the blood in nephritis is entirely insufficient to account for the amount of nitrogen retention. Despite this fact, the degree of retention of the various waste products affords an excellent index of the severity of the condition, and probably a fair index of the retention in the late stages of the disease, particularly as regards the uric acid and creatinin.

In the studies of the above named authors it was soon noted that high uric acid estimations were often found without any other retention, while creatinin appeared to be retained only in the last stages of the disease. It was also observed that the kidney was able to concentrate the creatinin 100 times, the urea 80 times, but the uric acid only about 20 times. It appears, therefore, that normally creatinin is the most readily, and uric acid the least readily, eliminated by the kidney, urea maintaining an intermediate position. It might be possible to offer the hypothesis, then, that the uric acid retention is the first to become evident, urea the next, and creatinin the last. This has proven to be true by ex-

perience.

Accordingly, a retention of uric acid should constitute one of the early signs of incipient interstitial nephritis, while a considerable creatinin retention should indicate a grave functional impairment of the kidney, and should be a valuable prognostic test.

Theoretically, the amount of the increase in the creatinin of the blood should be a safer index of the permeability of the kidney than either the urea or the uric acid, for the reason that creatinin on a meat-free diet is entirely endogenous in origin, and its formation (and elimination normally) very constant. Urea, on the other hand, is largely exogenous under normal conditions, and its formation, therefore, is subject to great fluctuations, the same being true in a measure of uric acid. The handicap of a high creatinin accumulation, the kidney is apparently never able to overcome. As already noted, a rise in the concentration of the uric acid of the blood appears to be an excellent early sign of chronic (interstitial) nephritis. As a prognostic test, the blood creatinin has been found to be of very great value. It appears to be of more value than the phenolsulphonphthalein test, which remains continuously negative, while the changes in the blood creatinin clearly show the patient's condition.

The normal findings are uric acid, from 2 to 3 milligrams per 100 c.c. of blood, and creatinin, from 1 to 2.5 milligrams per 100 c.c. of blood. Blood creatinin estimations of 5 milligrams or more per 100 c.c. of blood, practically indicates a fatal termination in a comparatively short period.

References: Chase and Meyers: Journal A. M. A., 1916, lxvii, 933; Meyers and Lough, Arch. Int. Med., 1915, xvi, 536; Fine, Postgraduate, 1914, xxix, 440.

Methods for the Estimation of the Creatinin and Uric Acid in the Blood.—The following methods for the creatinin determination are those given by Meyers and Lough:

"In most of our work the Duboscq colorimeter has been utilized for the estimation of the creatinin and creatin somewhat after the technic by Folin. About 10 c.c. of blood are drawn directly from a vein into a small bottle containing a little powdered potassium oxalate or 5 drops of a 20% solution to prevent clotting. Six c.c. of the well mixed blood are treated with 24 c.c. (4 volumes) in a 50 c.c. centrifuge tube. After the corpuscles have been laked, about 1 gram of dry picric acid is added, and the mixture stirred at intervals with a glass rod until it is a light yellow. When the protein precipitation is complete, the tube is centrifuged and the supernatant fluid filtered through a small 7 cm. filter paper. From 17 to 21 c.c. of filtrate are usually obtained (5 c.c. of which are removed for the creatin estimation and 3 c.c. for the sugar estimation, if these determinations are to be carried out). To 10 c.c. of the filtrate is added 5 c.c. of 10% sodium hydroxide, and a similar amount of alkali added to 10 c.c. of standard creatinin in saturated picric acid (containing 0.2, 0.5 or 1.0 mg. creatinin to 100 c.c. of picric acid). Creatinin may now readily be prepared perfectly by the admirable method of Benedict. A standard solution of this creatinin, 1 mg. to 1 c.c., is kept in 0.1 N hydrochloric acid. From this we have prepared a stock solution in picric acid, 5 mg. to 100 c.c. by diluting 5 c.c. to 100 c.c. with saturated picric acid. By pipetting 0.4, 1.0 or 2.0 c.c. of this solution into 10 c.c. graduates with a Mohr pipette and diluting to the mark, standards of

the above strength are prepared. For the Duboscq colorimeter the standard prism can conveniently be set at the 15 mm. mark.

"The estimation of the creatinin may likewise be carried out with the use of the Autenrieth-Konigsberger colorimeter of Hellige. In this case less blood is necessary. Two c.c. are treated in a cylindrical centrifuge tube with 8 c.c. of water and other manipulations as above. For the determination proper, 0.1 c.c. of 10% sodium hydroxide are added to 2 c.c. of the picric acid filtrate in a small test tube. Simultaneously 1 c.c. of the alkali is added to 20 c.c. of a saturated solution of picric acid containing 1.5 mg. creatinin to 100 c.c. to serve as a standard for the wedge. At the end of 10 minutes the wedge is filled with the standard, the cup with the unknown, and readings made. Although it is well to calibrate a wedge for a given instrument, the following formula, in which R represents the colorimetric reading and 5 the dilution, will suffice:

$$89\text{-R} \times 0.0179 \times 5 = \text{mg. creatinin calculated per 100 c.c. of blood."}$$

References: Folin, O.: Jour. Biol. Chem., 1914, xvii, 457; Benedict, S. R.: Jour. Biol. Chem., 1914, xxiii, 183.

The following methods for the determination of the uric acid in the blood are those given by Fine:

"The blood is drawn from a vein into a 2 or 3 oz. wide mouth bottle containing about 10 drops of a 20% solution of potassium oxalate to prevent clotting. The amount of blood to be used for the determination varies with the concentration of uric acid. For a concentration of 2 mgms. per hundred grams of blood, it is convenient to employ 25 c.c. of blood. This amount is pipetted into a casserole of 375 c.c. capacity and

5 volumes of N/100 acetic acid * added. The mixture is heated first on a boiling water bath and then carefully over a flame with constant stirring, until a drop suspended from a stirring rod appears water clear. At this stage it is filtered through a folded hardened filter paper into a cylinder, the coagulum returned to the casserole, treated with 150 to 200 c.c. boiling water, and filtered directly into the cylinder containing the first filtrate. If practically water clear, the combined filtrates may at once be evaporated to a small volume after the addition of 2 c.c. of glacial acetic acid. If not clear, clarification may readily be brought about by boiling with approximately one-half teaspoonful of a thin suspension of common talc powder. It is usually necessary to pass this through a filter paper twice in order to remove any finely divided talc. When evaporated to 5 or 10 c.c., the material is transferred to a smaller casserole (150 c.c. capacity), employing a rubber tipped stirring rod and hot water to facilitate the removal of any material adhering to the sides of the casserole. The contents of the smaller casserole are now evaporated to about 1 c.c., and transferred quantitatively to a 15 c.c. centrifuge tube. This is then placed in a beaker containing boiling water to aid in flocking out of the suspended material. The clear fluid is obtained by centrifuging and decanting; the residue is shaken up with about 5 c.c. boiling water and the clear supernatant fluid * obtained by centrifuging, and decanting into the main portion. In order to render the material entirely protein-free, it is frequently necessary to return the fluid to the casserole, evaporate to 1 c.c. and repeat the process of removing the residual protein coagulum as already described. When completely protein-free, transfer to a 15 c.c. centrifuge tube, care being taken

to keep the volume under 10 c.c. The uric acid is then separated as follows:

"Add 2 drops of magnesia mixture,* 5 drops of 3 per cent. silver lactate solution, and enough strong ammonium hydroxide (15 to 20 drops) to dissolve the silver chloride, after which the silver-magnesium-purine compound is observed to flock out. After standing a few minutes, it is centrifuged, the supernatant fluid decanted, and the last drop removed from the rim of the tube with filter paper. To the precipitate in the tip of the tube are then added one or two drops of glacial acetic acid and the compact mass thoroughly agitated. Finally 5 to 10 drops of strong hydrogen sulphide water are added, the mixture shaken, and placed in a boiling water bath for about 10 to 15 minutes; that is, until an excess of hydrogen sulphide is driven off and no brown coloration is observed after the addition of one or two drops of a 0.5 per cent. lead acetate solution. The tube is removed from the water bath, permitted to cool, and preparations made for developing the color and comparing it with the standard.

"*Using the Duboscq Colorimeter.*—One c.c. of a standard solution of uric acid, containing one mgm. uric acid per c.c.,* is placed in a 100 c.c. cylinder by means of an Ostwald-Folin pipette. To both the standard and to the contents of the centrifuge tube, containing the uric acid to be determined, are added 2 c.c. of the Folin-Macallum phosphotungstic acid reagent.* To the standard cylinder are then added 20 c.c. of a saturated solution of sodium carbonate; the material in the centrifuge tube is then poured into a 50 c.c. cylinder and treated with a like amount of sodium carbonate, the latter being first placed in the emptied centrifuge tube to wash into the cylinder any trace of uric acid. The standard is then made up to the 100 c.c.

mark and the unknown to the 50 c.c. mark. Both are shaken and the color at once compared in the colorimeter. It is convenient to set the standard cup of the Duboscq at 10 mm. The concentration of uric acid per 100 c.c. can be calculated from the formula $\frac{10 \times V}{R \times W}$, in which 10 represents the depth in millimeters of the standard, V represents the volume to which the unknown was diluted, R the reading in millimeters of the unknown, and W the weight or volume of blood employed for the analysis. It is desirable to develop the colors in such a manner that both the standard and the unknown will be as nearly as possible of the same intensity. The amount of blood used for the analysis and the volume to which the color is finally diluted are selected to meet this condition. The following will illustrate the relation between volume of blood used and final dilution of colors for bloods of various concentrations of uric acid in order to obtain concentrations identical in intensity with the standard.

Volume of Blood	Uric Acid Concentration of Blood	Final Dilution
c.c. or grams	mgms. per 100 c.c.	c.c.
25	1	25
25	2	50
25	3	75
25	4	100
10	1	10
10	2	20
10	3	30
10	4	40

"When using such small volumes as 10 c.c. of blood with only a normal concentration of uric acid, special care must be taken to keep the volume of the de-pro-

teinized fluid as low as 5 or 6 c.c. before precipitating the uric acid as the silver-magnesium-purine compound. In making up the colors of such final volumes as 10 to 50 c.c., the amount of Folin-Macallum reagent and sodium carbonate should be properly varied. Thus for a final volume of 25 c.c., 1 c.c. of the Folin-Macallum reagent and 10 c.c. of the saturated sodium carbonate is sufficient. It is of course not always possible in an initial examination to bring about the ideal conditions for color comparisons, since the concentration of the uric acid is not known; and it may, therefore, be necessary to make a second determination in order to obtain a wholly satisfactory result.

"*Using the Hellige Colorimeter.*—In comparing the colors a standard of double strength is employed for

TABLE FOR THE ESTIMATION OF URIC ACID WITH THE HELLIGE COLORIMETER.

Colorimetric reading	Uric acid mgms. per 100 c.c. dilution	Colorimetric reading	Uric acid mgms. per 100 c.c. dilution	Colorimetric reading	Uric acid mgms. per 100 c.c. dilution
20	1.67	40	1.28	60	0.88
21	1.65	41	1.26	61	0.86
22	1.63	42	1.24	62	0.84
23	1.61	43	1.22	63	0.82
24	1.59	44	1.20	64	0.81
25	1.57	45	1.18	65	0.79
26	1.55	46	1.16	66	0.77
27	1.53	47	1.14	67	0.75
28	1.51	48	1.12	68	0.73
29	1.49	49	1.10	69	0.71
30	1.48	50	1.08	70	0.69
31	1.46	51	1.06	71	0.67
32	1.44	52	1.04	72	0.65
33	1.42	53	1.02	73	0.63
34	1.40	54	1.00	74	0.61
35	1.38	55	0.98	75	0.59
36	1.36	56	0.96	76	0.57
37	1.34	57	0.94	77	0.55
38	1.32	58	0.92	78	0.53
39	1.30	59	0.90	79	0.51

Note: Recently, Leitz, Inc., New York, N. Y., has put on the market several types of micro-colorimeters. These instruments require less blood, and give results accurate enough for clinical purposes.

the wedge, e.g., the 1 mgm. is diluted to only 50 c.c. By consulting the accompanying table the uric acid corresponding to the colorimeter reading is obtained, from which the concentration of uric acid may be readily calculated.

"* N/100 acetic acid is prepared by diluting 0.6 c.c. glacial acetic acid to one liter.

"If at this point the suspended material does not settle readily, one or two drops of a very dilute solution of colloidal iron (One drop of a 5 per cent. solution diluted to 25 c.c.) may be added.

"Magnesia mixture is prepared as follows: 35 grams $MgSO_4$ and 70 grams NH_4Cl in 280 c.c. distilled water. Add 140 grams concentrated NH_4OH and thoroughly mix.

"A solution of 1 mgm. uric acid per c.c. is easily prepared by dissolving in a 100 c.c. beaker 100 mgms. Kahlbaum's uric acid in 50 c.c. of a 1 per cent. piperazine solution. Transfer quantitatively to a 100 c.c. volumetric flask and make up to the 100 c.c. mark. One c.c. of such a standard solution contains one mgm. uric acid. It should be made up fresh at least every week.

"The Folin-Macallum phosphotungstic acid reagent is prepared by boiling 100 grams Kahlbaum's sodium tungstate and 80 c.c. of 85 per cent. phosphoric acid in 750 c.c. of distilled water for two hours in a liter flask provided with a funnel to prevent evaporation. The reagent is finally made up to a volume of 1,000 c.c. with distilled water."

4. Estimation of Blood Coagulation Time as Test of Renal Function

Bachrach-Tittinger Test.—In cases of renal insufficiency, such cases as those in which it is claimed that ures for) the coagulation time has been found de-
ures for δ) the coagulation time has been found delayed. This delay is supposed to be connected with salt retention in the plasma. A rather large amount of blood is required according to the original technic of the originators (20 c.c.). The test is not credited with much value.

For method of estimating blood coagulation time see page 41.

5. Cryoscopy of Blood as Test of Renal Function

Cryoscopy of the Blood.—This method has been employed by some investigators on the principle that under conditions of renal impermeability the waste products which fail to be eliminated in the urine will accumulate in the blood, thereby increasing the molecular concentration. This means, of course, a lowering of its freezing point.

The technic of estimating cryoscopy of the blood is not different from that applied to the urine, which has been already described (v. page 86). This method has not come into general use, however, and for this reason it will not be further considered here.

III. STUDY OF THE ELIMINATION OF FOREIGN SUBSTANCES BY THE KIDNEY, AS CRITERION OF KIDNEY FUNCTION

This category of tests may be divided into two parts:

1. Miscellaneous Chemical Substances:
   a. Potassium iodide.
   b. Phloridzin.
   c. Hippuric acid.
   d. Lactose.
2. Elimination of Dyes by the Kidney.
   a. Methylene blue.
   b. Indigo carmine.
   c. Phenolsulphonephthalein.

1. *Tests with Miscellaneous Chemical Substances*

a. *The Potassium Iodide Test.*—This was one of the first chemical substances applied to the estimation of renal function since it was suggested by Duckworth as long ago as 1867.[22]

Potassium iodide is rapidly absorbed from all the mucous membranes.[23] It is absorbed unchanged and appears quickly in the excretions. Only a few minutes normally elapse before it can be demonstrated in the urine. According to the investigations of Quetsch,[24] Roux [25] and Studeni [26] it appears at any time from 9 to 18 minutes after doses of 1 to 3 grams have been swallowed. The greater part of the iodide ingested is excreted in the urine. Some, however, escapes in the saliva and other secretions.

Iodide is rapidly excreted, since 65-80% of the amount ingested is eliminated in 24 hours. Several

[22] St. Barthol. Hosp. Rep., 1867, III, 216.
[23] Cushing Pharmacology, 5 Ed., 1910, 510.
[24] Berl. klin. Wchnschr., 1884, XXI, 353.
[25] Thèse de Paris, 1890, no. 248.
[26] Inaugural Dissertat., Zurich, 1897.

investigators have reported the exact time required for complete elimination to take place. According to Antem,[27] .5 gram requires 40 hours to be excreted, and Schlayer and Takayasu,[28] and Monakow [29] state that they found the same amount required 48 hours to eliminate. Schlayer concluded from his studies that the demonstration of iodide in the urine beyond 60 hours after its administration may be considered delayed, therefore a pathological excretion.

Schlayer and his followers endeavored to fix as they did with sodium chloride the exact locus of elimination for iodide in the kidney. They believed that iodides are excreted by the tubular epithelium. They also contended that the elimination of iodide is not delayed in passive congestion (cardiac) while it is delayed in chronic tubular nephritis. These suppositions have neither of them been substantiated by subsequent investigations.

Rowntree and Fitz in their experience with the iodide test have found it to vary markedly and they believe that the observation of excretion time of potassium iodide as a test of renal function is unreliable.

Technic of Iodide Test.—.5 gm. (7½ grains) of potassium iodide is given in solution by mouth in the morning on arising. The urine is collected at the end of 48 hours and every four hours thereafter and tested for iodide until a negative result is obtained.

One of the simplest and best qualitative tests for iodide in the urine is that of Sandow. The test is made by taking 30 c.c. of urine, 2 c.c. of 2% solution of sodium nitrate and 2 c.c. of dilute sulphuric acid,

[27] Arch. f. Pathol. u. Pharmacol., 1902, XLVIII.
[28] Deutsch. Arch. f. klin. Med., 1911, CI, 354.
[29] Deutsch. Arch. f. klin. Med., CII, 248.

adding chloroform and shaking. A purplish or violet color appears in the chloroform if iodide is present.

b. *The Phloridzin Test.*—Von Mering[30] discovered the fact that the injection of the glucoside phloridzin into animals, produces a glycosuria without a hyperglycemia, thus proving that the conversion is a vital act of the renal parenchyma. Achard and Delamare[31] built upon this fact a method of testing the functional capacity of the kidney.

Technic of Phloridzin Test.—.005 gm. of phloridzin in fresh aqueous solution is injected hypodermically. At 15-minute intervals the urine collected by catheter or voided spontaneously is examined for sugar. The maximum excretion takes place normally in an hour and disappears in 2 or 3 hours.

c. *The Hippuric Acid Test.*—It has been long known that benzoic acid or benzoates are eliminated by the kidney as hippuric acid which is synthesized in the kidney from benzoic acid and glycocoll. Altho the fact has been used as a basis for testing kidney function the results have been disappointing and the method has been abandoned.

d. *The Lactose Test.*—Voit[32] was the first to demonstrate that lactose is eliminated by the healthy kidney following its subcutaneous or intravenous injection. De Bonis[33] claimed that the elimination takes place exclusively in the glomerulus.

[30] Centralbl. f. med. Wissensch., 1885, 531.
[31] Bull. et Mem. Soc. Méd. d. Hôp. de Paris, 1899, 379.
[32] Deut. Arch. f. klin. Med., 1897, LVII, 545.
[33] Giorn. intern. d. scien. Med., 1907, XXIX, 446.

Lactose was suggested as a means of estimating renal functions in 1911 by Schlayer and Takayasu,[34] who with their co-workers studied the question of renal function in experimental and clinical nephritis. These workers studied the elimination of lactose, potassium iodide, salt and water, dividing the nephritides into vascular and tubular varieties with subdivisions.

Schlayer believed that the elimination of lactose being exclusively, as he thought, a glomerular function could be taken as an index of the vascular functioning power of the kidney. Lactose being a foreign substance that is not found in the organism would not be influenced in its elimination by extrarenal factors, and should therefore be an ideal criterion of glomerular function, any delay in its passage through the kidney indicating glomerular insufficiency.

Following Nussbaum's [35] technic of obtaining in the frog an exclusively tubular secretion from the kidney by artificially excluding the glomerular secretion, Rowntree and Fitz [36] concluded that the tubular epithelium can secrete a certain amount of lactose, hence its elimination is not exclusively a function of the glomerulus. But they concluded also from their clinical experiments with lactose in the study of renal function in practice and also from some studies they have made in experimental passive congestion, that the mechanism of lactose excretion differs essentially from that of phthalein, salt, indigo carmin, etc., and that estimations of lactose excretion may be looked upon as a satisfactory index of the vascular, if not exclusively the glomerular function of the kidney.

Technic of Lactose Test.—Two and five-tenths (2.5)

[34] Deutsch. Arch. f. klin. Med., 1911.
[35] Pflüger's Arch. f. d. ges. Physiol., 1878, XVI, 179; XVII, 580.
[36] Archives of Int. Med., 1913, XI, 258.

gms. of chemically pure lactose are dissolved in 25 c.c. of freshly distilled water, placed in small cotton stoppered Erlenmeyer flasks and pasteurized for four hours for four successive days at 75 to 80° C. By this method the dose injected amounts to a little over 2 gms. lactose in 20 c.c. of water. A fresh solution is used for each injection and a careful technic for intravenous injection carried out.

Following the injection there are usually no constitutional disturbances altho occasionally there may be some headache malaise or even chill followed by fever.

The urine is collected four hours after the injection and every hour or two hours after for twelve hours. Each specimen is tested for sugar by Nylander's reagent, using the same amount of urine, solution and length of time for boiling. Polarimetric readings may be made.

The normal excretion time for this amount of lactose is four to six hours. The time required for secretion is the main point. Over six hours is delayed excretion.

(Nylander's reagent consists of Rochelle salts, 4 gms. dissolved in 100 c.c. of 10% NaOH (sp. gr. 1.015); warm and saturate with bismuth subnitrate (about two grams are necessary). When cool, filter and keep in a dark bottle. The solution remains permanent for years.)

2. *Elimination of Different Coloring Matters or Dyes as a Measure of Renal Function. Urinary Chromoscopy*

There are three of these tests used at the present time, namely:

1. The Methylene Blue Test.

2. The Indigo Carmin Test.
3. The Phenolsulphonphthalein Test.

Other coloring matters such as rosanilin, fuchsin, etc., have been suggested and employed at different times for estimating renal function, but with the exception of the three named they appear to have fallen into disuse at the present time.

Numbers 2 and 3 are most extensively employed, namely, indigo carmin which is used particularly in Europe and phenolsulphonphthalein which is by far the most popular colorimetric test in this country.

Rosanilin (sodium trisulphate) was introduced by Lepine.[37] One c.c. of a 1% solution is injected hypodermically. The dye appears normally in the urine in less than half an hour, total elimination requiring twenty-four hours. The test has never attained wide use, probably because of the greater success attending the use of phenolsulphonphthalein according to the method of Rowntree and Geraghty (q. v.).

a. *The Methylene Blue Test.*—The introduction of methylene blue as a test for renal function is credited to Achard and Castaigne.[38] These authors gave the drug intramuscularly, using 1 c.c. of a 5% solution. Later Czyhlarz and Donoth[39] recommended it by mouth in ¼ grain dose. The drug is rapidly eliminated in the urine, appearing therein in about fifteen minutes as a colorless chromogen, as demonstrated by Voisin,[40] which may be shown by boiling the urine after addition of acetic acid. Normally the color itself appears in the urine in half an hour. The

[37] Lyon Médical, 1898.
[38] Bull. et Mem. Soc. Méd. d. hôp. de Paris, April, 1897, 637.
[39] Wien. klin. Wchnschr., XXIV, 1899, 649.
[40] Gaz. Hebd. Méd., 1897, 493.

excretion of both forms continues for 36-48 hours, but even in health the time may be very much prolonged.

The authors of the test recommended that the time of first appearance, time of maximum intensity of excretion and time required for total excretion should be noted.

Diminished renal permeability was thought to delay or prolong all three. Various observers confirmed these suppositions. In some cases of chronic interstitial nephritis the elimination was found to be prolonged for two weeks. Various modifications of the type of elimination under pathological conditions were described, consisting of remittances or intermittances of excretion.

Later observers noted that elimination is not delayed in all forms of kidney disease and that the elimination under certain circumstances might be normal or even accelerated.

Attempts to measure quantitatively the elimination of methylene blue were made, one of which described by Rowntree and Geraghty will be mentioned under technic.

The methylene blue test was later applied to diagnosis in surgical diseases of the genitourinary tract and was at one time considered the best test available for estimating the functional capacity of one or both kidneys. Walker showed that the elimination is retarded in lower urinary tract obstructions as in certain types of prostatic hypertrophy. Casper made similar observations.

Methylene blue produces some pain when given subcutaneously and occasionally when given intramuscularly. This of course is a drawback though perhaps not a serious one. Another and greater drawback is its prolonged elimination necessitating a large number of

urine examinations. A third objection is the difficulty of accurate colorimetric estimations.

Finally, the methylene blue test is imperfect in that part of the substance is converted into a colorless chromogen in the body secreted as a leucobase and therefore does not contribute to the color results in the urine. Only 50% of the drug is normally passed out in the urine.

It has not been demonstrated with certainty in what part of the kidney the elimination of methylene blue takes place.

In disease of the tubular epithelium that is in the chronic parenchymatous nephritis, methylene blue is quickly and completely eliminated; in interstitial nephritis, the elimination is delayed.

The duration of elimination is diminished in parenchymatous and increased in interstitial nephritis. A cyclic, polycyclic or intermittent elimination of the coloring matter has been said to indicate several different conditions: disturbance of kidney innervation, hepatic insufficiency, interstitial nephritis and pyohydronephrosis.

Up to the time of the advent of the phenolsulphonphthalein test, the methylene blue test was the most extensively used method of determining renal permeability. At the present time, certainly in America, the Rowntree-Geraghty test has quite superseded it.

Technic of Methylene Blue Test.—After urination 1 c.c. of a 5% solution of methylene blue is injected intramuscularly. A sterile catheter may be introduced into the bladder or the patient may empty the bladder in 15 minutes if possible to determine the presence of the leucobase or chromogen. This is done by boiling the specimen and adding a few drops of acetic acid. A greenish color denotes the presence of the chromogen.

At the end of half an hour the bladder should be emptied spontaneously or by catheter and the urine examined for color. A greenish blue color denotes the presence of the dye.

As above mentioned, the time of appearance, time of maximum intensity and time required for total elimination (disappearance of color) should be noted.

Quantitative Estimation.—Before administration of the drug the urine is collected for some time and kept. The methylene blue is given in the usual manner, the urine collected for as long a time as necessary, all chromogen being converted into dye. An equal quantity of urine previously collected is taken, to which is added from a burette, drop by drop, a sufficient quantity of a solution of methylene blue of known strength until the colors are alike. Compare against a white background. From the quantity of methylene blue used, the amount of coloring matter may be estimated.

b. *The Indigo Carmine Test.—Volcker-Joseph Test.*[41]—The dye was first used by Heidenhain in his famous investigations into the physiology of the kidney. He believed that this substance is eliminated exclusively by the epithelial cells of the convoluted tubules.

Indigo carmin possesses the advantages over methylene blue that the quantity required for the test is completely eliminated through the kidney and that no leucoderivative is formed in the tissues.

After the intramuscular injection of .08 gm. to .16 gm. of indigo carmin, elimination begins in 6 to 8 minutes if the kidney is normal. The intensity of the color will give some idea of the concentrating power of the

[41] Münch. med. Wchnschr., 1903, 2081.

kidney, that is to say, the water resorbing power of the tubules, and consequently, its capacity to produce a urine of high molecular concentration.

A polycyclic or intermittent elimination is said by Blum to indicate intermittent hydronephrosis. In strongly alkaline urine the dye may be discolorized. In parenchymatous nephritis, the elimination of indigo carmin may be normal. In interstitial nephritis the elimination begins later than normal, is diminished in quantity and much prolonged. The delayed elimination indicates a diminished reaction power of the kidney, the diminished elimination a loss of water resorbing power, a hyposthenuria, a loss of the concentrating function of the organ, while the long duration of elimination indicates a general loss of secreting power as always accompanies the sclerotic kidney.

The indigo carmin test has been quite extensively used, especially in Europe, in testing the functional capacity of the single kidney by ureteral catheterization and in general functional testing of the kidney. It is considered superior to methylene blue because of more rapid elimination. But in this respect, as in others, it is inferior to phenolsulphonephthalein, which substance, since its introduction for this purpose by Rowntree and Geraghty, has become the most extensively used chromoscopic test, at least in this country.

Rowntree and Geraghty consider the indigo carmin test of more value than the methylene blue test because of its more rapid appearance in the urine, but that it is less adapted to functional work than phenolsulphonephthalein. This opinion is now shared by most other workers in this field.

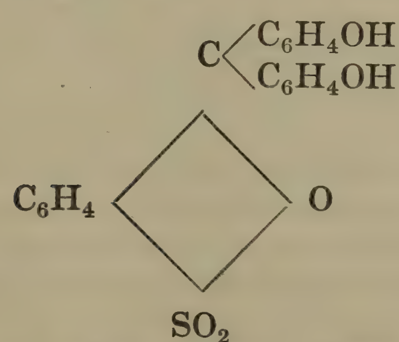
Technic of Indigo Carmin Test.—A 4% solution is made. Twenty c.c. of this solution are injected into the muscles usually of the gluteal region. There is some

pain produced by the injection. In 10 to 15 minutes the urine is collected. In normal persons it is tinged greenish blue in this time. Excretion is usually complete in 24 hours, but practically the greater portion escapes in 12 hours. The color of the dye in the urine does not lend itself well to colorimetric estimation. In this respect it resembles methylene blue. Purulent urine decolorizes indigo carmin. It is estimated that not more than 25% of the amount injected finds its way out through the kidneys. The fate of the balance is unknown.

c. *The Phenolsulphonephthalein Test. Rowntree-Geraghty Test (The Red Test).*—The phenolsulphonephthalein test of kidney function had its origin in the pharmacological researches of Abel and Rowntree, upon the phthaleins generally. Of all the phthaleins studied by these investigators, phenolsulphonephthalein stood out in striking contrast with all others because of the fact that it is almost exclusively eliminated by the kidney.

This fact suggested its use as a test of renal function to Rowntree and Geraghty and their first communication upon this subject appeared in July, 1910.[42]

Phenolsulphonephthalein has the following formula:



[42] Jour. Pharmacol. and Exp. Therap., I, 1910, 579.

It was first made by Remsen.[43] The substance is a red crystalline powder, partly soluble in water, the solution when alkaline being red, becoming more purple as the alkalinity is increased.

Abel and Rowntree in their pharmacological investigations of the phthalein group showed that the substance appears in the urine after administration by mouth in one to one and a half hours and after subcutaneous injection in about 10 minutes. They found that after fair-sized doses (1 gm.) the drug appears in the bile, is passed into the intestine, there reabsorbed, and, except for a mere trace, is excreted wholly by the urine.

Phenolsulphonephthalein is practically non-toxic. When a dose of .006 gm. is injected subcutaneously, 40-60% of this quantity is recovered in the urine during the first hour after injection. From 15-25% more is recovered in the second hour, making a total excretion for the first two hours following the injection of 60-85%.

Normally when the urinary flow is free, the dye appears in the urine in 5 to 10 minutes after injection. The maximum excretion appears in 15 to 20 minutes. This density of excretion continues an hour to an hour and a half. The elimination then begins to diminish. At the end of the first hour the pink color on addition of alkali is slight, and after the expiration of two hours excretion is practically complete.

In acute nephritis, Rowntree and Geraghty [44] found a diminished excretion of phthalein in two out of three cases. In parenchymatous nephritis there was a marked diminution of excretion in seven out of eight cases. In chronic interstitial nephritis a low output was encoun-

[43] Amer. Chem. Jour., VI, 280.
[44] Jour. Phar. and Exp. Ther., I, 1910, 656.

tered in all the cases experimented upon, 10 in number.

These results concerning the lowered excretion of phenolsulphonephthalein in the nephritides have as a general thing been entirely corroborated in subsequent investigations by many different observers.

In their first researches Rowntree and Geraghty made over two hundred functional tests in one hundred and fifty persons. To them the phenolsulphonephthalein seemed to possess advantages over all other functional tests, these advantages consisting chiefly in the following points:

1. The early appearance of the dye in the urine and its rapid and complete elimination by the kidney.
2. The accuracy and simplicity of quantitative estimation of the drug in the urine.

They showed by these researches that the permeability of the kidney for phenolsulphonephthalein is decreased in both parenchymatous and interstitial nephritis, the decrease being most marked in the latter form.

Further than this they showed that the test is of value to the surgeon in determining the true condition of the kidney, in cases with prostatic obstruction. In such cases the authors believed the phenolsulphonephthalein test to be of greater service than urinalysis or nitrogen estimations and that the use of the test in cases of obstruction in the lower urinary tract prior to operations would disclose the necessity of preliminary treatment. Finally they pointed out that the test lends itself and is well adapted to unilateral estimations of the functions of the separate organs in conjunction with ureteral catheterization.

In surgical cases with urinary obstruction, the authors contended that when the phthalein excretion is delayed beyond twenty-five minutes and the output for the first hour is below 20% the operation may prof-

itably be postponed until treatment by drainage has improved conditions, such improvement being shown by an increase in the elimination of phthalein at a subsequent time.

Technic of Phenolsulphonephthalein Test.—Twenty minutes to half an hour before giving the test the patient is given 200 to 400 c.c. of water to insure diuresis. The bladder is catheterized or completely emptied. The time being noted, 1 c.c. of a solution of the drug is injected into the lumbar muscles. The solution is prepared as follows: .6 gm. phenolsulphonephthalein and .84 c.c. $\frac{2}{n}$ NaOH are added to .75% NaCl solution. Add two or three drops of $\frac{2}{n}$ NaOH. The color becomes Bordeaux red and the solution is non-irritant.

The urine is passed into a test tube containing a drop of 25% NaOH and the time of appearance of the first pinkish color noted.

If there is no urinary obstruction the catheter is not necessary after the appearance of the color, and the patient may then retain the urine and urinate at the end of one hour in one receptacle and again at the end of the second hour in another.

A rough estimate of the time of the appearance of the drug in the urine may be gained by having the patient urinate, frequently, a small amount without the catheter. In prostate cases it seems better to keep a catheter *in situ*. If this is done the catheter may be corked and this is removed at the end of the first and second hours.

Each sample of urine is measured. Twenty-five per cent. sol. NaOH is added to make the color maximum.

The urine is usually yellow or orange and becomes deep purple on addition of the alkali. The solution is put in a liter flask and diluted with distilled water to make a quart. This is thoroughly mixed and a portion is filtered and compared with a standard in a colorimeter.[45] The standard solution consists of .003 gm. phenolsulphonephthalein (½ c.c. of solution used for injection) diluted to 1 liter and made alkaline with a few drops of 25% NaOH. The test solution retains its fine purplish color for a week or more.

The colorimeter contains a wedge-shaped cup which is filled with the standard solution. The rectangular cup is filled with the solution to be tested. The wedge-shaped cup is manipulated by a screw until the color fields are identical. The percentage is read off on the indicator scale.

Technic of the Phenolsulphonephthalein Test as Applied to Estimation of the Function of the Individual Kidney.—Twenty minutes previous to the application of the test the patient is given 600 to 800 c.c. of water to provide a free flow of urine. The ureters are catheterized, a special catheter being recommended, namely, the flute end catheter of Albarran No. 6 or No. 7. The catheters are passed four inches into the ureters. The cystoscope is withdrawn, leaving the catheters *in situ*. A small urethral catheter is passed into the bladder to empty that organ and detect later leakage. The other details of the test are similar to those of the ordinary technic (q. v.).

In September, 1911, Geraghty and Rowntree [46] made

[45] The colorimeter used by Rowntree and Geraghty is a modification of the Autenrieth-Konigsberger instrument. This can be obtained from Hynson and Westcott, Balto., Md., who also supply convenient ampoules containing .006 gm. in each c.c. of phenolsulphonphthalein.

[46] Jour. Amer. Med. Assn., 1911, LVII, 815.

a report of their previous experience with the sulphonephthalein test and reiterated their first opinion that the test devised by them appeared to possess distinct advantages over all other methods of examining renal function. The reasons upon which this opinion was based have been given above.

They concluded that the sulphonephthalein test will enable the clinician to determine quantitatively the amount of functional derangement of the kidneys in his nephritis cases, whether of the acute or chronic type. That in cardiorenal cases the test will show exactly to what extent the kidney is involved. That the test is of special value in the diagnosis of uremia from other conditions which may simulate it and also to foretell in many cases an impending uremia before the appearance of indubitable clinical signs.

The authors reiterated their confidence in the value of this test in cases of urinary obstruction, it being in their judgment superior under these circumstances to measuring the urinary quantity, and to urea or total nitrogen estimations.

In many surgical cases studied by them in the genito-urinary clinic of Young at Johns Hopkins, they found that separate studies of unilateral kidney function revealed more accurate and dependable information than any other method of examination.

In a third and very complete report of their phenolsulphonephthalein test of kidney function published in 1912, Rowntree and Geraghty studied carefully the influence on the rate of excretion, of the various methods of administration, subcutaneous, intramuscular and intravenous. They concluded from their researches that intramuscular injection into the lumbar region is the method of choice.

Studying the influence of various diuretics upon the

excretion of sulphonephthalein the authors found that while under the conditions of animal experimentation, some slight increased activity was caused by certain stimulating diuretics like caffein, yet clinically these substances do not affect the phthalein output.

The route of phthalein through the kidney was investigated and it was demonstrated that the drug is excreted chiefly by the uriniferous tubules and the smaller remainder by the glomerulus.

In nephritis of all types the output of phthalein was found diminished, the diminution of excretion being apparently in proportion to the amount of damage to the kidney structure. So that the test is of considerable value from a diagnostic and prognostic standpoint since the amount of functional incapacity is revealed.

In those cases in which the heart and kidney are both affected (the so-called cardiorenal cases) the test was found useful in determining just what proportion of the trouble could be referred to the heart disease and what to the renal lesions.

In uremia the test proved in the hands of its authors of value in differentiating uremia from conditions simulating it. In certain cases, when no clinical evidence of the imminence of uremia was present, a very low phthalein output frequently enabled the authors to foresee the danger.

From a surgical standpoint the earlier opinions held by Rowntree and Geraghty as to the utility of the test in cases of urinary obstruction were completely corroborated by their subsequent work.

According to them the test is more dependable than estimation of urinary output, total solids, urea or total nitrogen, in indicating to the surgeon the propriety of operation or the institution of preliminary treatment, in contemplated nephrectomy and prostatectomy.

Finally their studies tended to show that no other test is so adaptable to the examination of unilateral kidney function to determine the relative amount of work performed by each organ separately.

The medical and surgical aspects of the phthalein test will be further developed later on under the general summary of renal function tests in their medical and surgical aspects. (*v.i.*)

Since the introduction of the phenolsulphonephthalein test for kidney function in July, 1910, a very considerable literature upon the subject has appeared. It is very striking how few are the criticisms and how numerous are the encomiums which have been passed upon the test of Rowntree and Geraghty. It might almost be said that, in this country at least, the opinion of those who have used it is unanimously favorable and tends to corroborate in every particular the claims which were advanced for it by its authors.

Clinical and experimental corroboration of the sulphonphthalein test have been given by the publications of Austin and Eisenbrey,[47] Boyd,[48] Cooke,[49] Sehrt,[50] Lance,[51] Sanford,[52] Behrenroth,[53] Frank,[53] Bonn,[54] Erne,[55] Mouriquand,[56] Lohnstein,[57] Frothingham, Fitz, Folin, Denis,[58] Christian, Janeway, Cabot,

[47] Jour. Exper. Med., 1911, XIV, 367; 462.
[48] Jour. Amer. Med. Assn., 1912, LVIII, 620.
[49] Providence Med. Jour., 1912, XIII, 118.
[50] Centralbl. f. Ch., 1912, XXXIX, 2, 1121.
[51] Gaz. d. hôp. de Par., 1912, LXXXV, 32.
[52] Cleveland Med. Jour., 1912, XI, 763.
[53] Ztsch. f. Exp. Pathol. u. Therap., 1913, XIII, 72.
[54] Jour. Ind. State Med. Assn., 1913, VI, 154.
[55] Münch. med. Wchnschr., 1913, LX, 510.
[56] Lyon Médicale, 1913, CXXL, 299.
[57] Allg. Med. Centr. Gtz., 1913, LXXXII, 591.
[58] Arch. Int. Med., 1913, vols. XI-XII; also Jour. Exper. Med., 1911, XIV, 366.

Dock,[59] Snowden, Thayer,[59a] and many others.

While many of these reports are extremely illuminating and important, it cannot be said that they have added anything noteworthy to the test itself, or to its indications, which fact is a strong testimony of the thoroughness and care with which the work had originally been performed by Rowntree and Geraghty before its publication.

From the standpoint of pure experimental corroboration, the work of Austin and Eisenbrey should be noted. These authors in 1911 studied the elimination of phenolsulphonephthalein as compared with the elimination of nitrogen and chlorides, in experimental nephritis in dogs set up by administering uranium, cantharidin, and potassium bichromate. They concluded, among other things, as a result of their researches, that a marked and early decrease in the elimination of phenolsulphonephthalein takes place in the experimental nephritides and that the phthalein test is the better indicator of renal function under the circumstances than total nitrogen or chloride elimination, which latter are more irregular and inconstant.

Other investigators have experimented along the same line, studying the phthalein elimination in experimental nephritis and their results have tended to corroborate the earlier researches. Perhaps the most recent contribution to this phase of the subject is that of Potter and Bell.[60] These authors have studied the phthalein elimination, also that of lactose and potassium iodide in experimental tartrate nephritis in rabbits.

It may be recalled that Underhill, Wells and Goldschmidt [61] discovered that the injection of racemic tar-

[59] Tr. Cong. Amer. Phys. & Surg., 1913, IX, 45.
[59a] Amer. Journ. Med. Sc., 1914, CXLVIII, 781.
[60] Amer. Jour. Med. Sci., CXLIX, 1915, 236.
[61] Jour. Exper. Med., 1913, XVIII, 322.

taric acid into rabbits produces a type of acute nephritis in which the great majority of the convoluting tubules become necrotic and the rest are fatty and granular. The glomeruli may be anatomically intact. In kidneys of this type it has been found that the excretion of phenolsulphonephthalein, likewise indigo carmin and methylene blue, is completely suppressed. The excretion time of lactose is over twice as long as normal, while that of potassium iodide is four times as long as normal, but both lactose and potassium iodide are excreted by this type of kidney. Potter and Bell suggest that their results appear to show that phthalein, indigo carmin and methylene blue are excreted exclusively by the tubules, while potassium iodide and lactose are at least partly excreted by the glomeruli.

In experimental chronic passive congestion of the kidney in animals, produced by compression of the vena cava and renal veins, Rowntree, Fitz, and Geraghty [62] found that the functional capacity of the kidney as judged by the phthalein output is reduced. The reduction, however, only occurs as the degree of passive congestion becomes marked.

Goldsborough and Ainley in 1910 [63] studied the renal function in pregnancy and the puerperium by means of sulphonephthalein, and concluded that even normal females in pregnancy eliminate less phthalein than non-pregnant. In the ninth month the power of elimination may be very low. The exact meaning of these facts is not known. This result was confirmed by Roth,[64] who also claimed that women with diseases of the genital tract were unsuitable for the test. These investigations of Goldsborough, Ainley and Roth have not been subse-

[62] Arch. of Int. Med., 1913, XI, 121.
[63] Journ. Amer. Med. Assn., 1910, LV, 2058.
[64] Berl. klin. Wchnschr., 1913, L, 1609.

quently developed. In this connection it may be mentioned that Pepper and Austin [65] reported in 1913 that occasionally cases of parenchymatous nephritis will show a quite prompt and fairly normal elimination of phthalein. In one of their cases there was a phthalein output of 67% for one hour, strongly suggesting hyperpermeability. Baetjer has also encountered similar cases. Such cases do not appear to represent the rule, however, and just what meaning is to be attached to these facts is at present unexplained.

A few suggestions for slight variations of technic in the test have been published but none of them appears to have been generally adopted. They are few in number and the most important may be given.

Fromme and Rubner [66] in 1912 suggested that the phthalein should always be given hypodermically and that the observation period should be extended to three hours. Keyes and Stevens [67] also recommend hypodermic injection when the ureters are to be catheterized. Bonn,[68] as a result of his experience, thought that the time of appearance of the dye in the urine is not of much importance except to determine the time for percentage estimation. He recommended the intravenous injection of the phthalein when the ureters are to be catheterized. He does not believe that the test will inform the surgeon when the patient can be operated on safely. He, however, states that, in his belief, the test is the best one yet devised for studying the renal function.

Fanz [69] suggests to add a quantity of the patient's urine obtained just before injecting the indicator, equal

[65] Amer. Journ. Med. Sc., 1913 n. s. CXLV, 254.
[66] Berl. klin. Wehnschr., 1912, XLIX, 1889.
[67] N. Y. Med. Jour., 1912, XCV, 1134.
[68] Indiana State Med. Assn. Jour., 1913, VI, 154.
[69] N. Y. Med. Jour., 1915, C, 1193.

in amount to the first hour's urinary output after the injection. The standard solution now will have approximately the same amount of urinary salts as the specimen solution, and the standard and urinary solution will equal each other in opaqueness and yellowish tint, making color comparison easy. To make up the standard solution, he uses 1 c.c. of the contents of an ampoule * of the phenolsulphonephthalein, adds the patient's urine (obtained before injecting the indicator) in amount to equal the first hour's urinary output after appearance of the drug. This mixture is alkalinized with 25 c.c. of 10% solution of potassium hydroxide, filtered, and sufficient distilled water is added to make 1000 c.c. This is the standard and contains 100% of the indicator in 1000 c.c.

The first hour's urinary output, after injection of 2 c.c. phenolsulphonephthalein, is now alkalinized with 25 c.c. of 10% potassium hydroxide and filtered. To this is added distilled water to make 1000 c.c. This is the test specimen. By diluting a unit of the standard, say 50-100 or 200 c.c., with distilled water until it matches the 1000 c.c. solution of the first hour's urinary output, the direct percentage of the indicator in the first hour's specimen can easily be estimated. Say 100 c.c. of the standard had to be diluted up to 500 c.c. before it matched the first hour's specimen dilution, then the standard would be five times as strong as the specimen, or the specimen would contain 20% of phenolsulphonephthalein. The second hour's output of phenolsulphonephthalein is estimated precisely like the first.

As a matter of fact it does not appear that any modifications of the original test method as described by Rowntree and Geraghty has contributed materially to its simplification or improvement, and for this reason

* Marketed by Hynson and Westcott.

the original method is usually followed.

Thayer and Snowden [70] have recently attempted to compare their results obtained by the test with the anatomical changes found in the kidneys at autopsy. They conclude, as the result of their quite extensive investigations, as follows:

In severe chronic nephritis there is always a low phthalein output. This rule, in their experience, has absolutely no exception. The phthalein output in cases of chronic nephritis diminishes steadily, according to them, until the terminal uremia, when it approaches zero, just prior to death.

In the passive renal congestion of heart disease, there is often a reduction in the elimination of phthalein, especially when the amount of decompensation of the heart is considerable. When these symptoms become ameliorated, the phthalein output increases.

If passive congestion of the kidney from heart disease is accompanied by concomitant chronic nephritis the output of phthalein is lower than in cases of uncomplicated congestion.

In one case of acute nephritis and one of amylosis the phthalein output was reduced.

In acute infectious diseases, during which cloudy swelling of the renal parenchyma occurred, there was found a considerable reduction of phthalein output.

From this study Thayer and Snowden concluded that the phenolsulphonephthalein test of Rowntree and Geraghty is a procedure of great diagnostic and prognostic value, especially in the study of chronic nephritis.

[70] Amer. Jour. Med. Sci., 1914, CXLVIII, 781.

GENERAL SUMMARY OF THE APPLICATION OF RENAL FUNCTION TESTS

This subject may be divided into two parts: 1. Renal Function Tests and Their Medical Application. 2. Renal Function Tests and Their Use in Surgery.

1. *Medical Application.*—A decade ago the burning question in renal pathology was whether the varying clinical findings in chronic nephritis could be divided into classes and each class correlated with certain definite histopathological findings in a kidney post mortem. Although no absolute answer to the question was reached, there is no doubt some progress was made. During the past decade, in line with the general departure of interest somewhat away from the anatomical toward the functional view in pathology, a serious attempt was made to divide the clinical symptomatology of nephritis into groups; to correlate these groups with certain definite types of renal involvement. Although this worthy attempt has not been entirely successful any more than its anatomical prototype which preceded it, some important facts have been learned which are of value from a practical as well as a theoretical standpoint. The method by which these advances have been gained is none other than the application of functional tests to a study of the different phases of renal activity.

Different portions of the glomerulo-tubular structure of the kidney have been supposed to possess selective secretory activities. It cannot be said that these selective activities are yet understood, so that at the present time there is no such thing as an exact topical diagnosis of the kidney functions.

Nor can it be said in any case that the results of the most complete and comprehensive functional examina-

tion will reveal with any certainty the anatomical changes which are present in the kidney. Certainly they will not reveal the extent to which the vascular, glomerular or tubular structures are involved in a given case, and will shed but little light upon the relative proportions of the changes.

Our chief clinical terms in renal pathology still remain as they were in the older tradition; we still speak currently of acute nephritis, chronic parenchymatous nephritis and chronic interstitial nephritis, but we are not surprised when the autopsy shows the extreme rarity of these arbitrary types and presents us with pathological pictures of such great complexity that it is no wonder that the intricate problem is not to be solved by the most skillful and painstaking ante-mortem examination.

Great hopes were aroused some years ago in this direction when it was discovered that experimental inflammation of the kidney may be set up in animals by the use of certain poisons [71] which make it possible to submit some of these difficult problems in renal pathology to experimental investigation. Although little has come of this work so far, it appears to be founded upon rational and scientific principles and the work must be enthusiastically encouraged to go on.

Schlayer, Hedinger, and Takayasu, in the Romberg Clinic at Tübingen, have applied themselves assiduously to working out the problems of pathological kidney function from an experimental standpoint. In this country the purely experimental method has yielded interesting results in the hands of Rowntree, Fitz, Geraghty, Christian, O'Hare, Folin, Karsner, Denis, Frothingham, Austin, Eisenbrey, Potter, Bell, and

[71] Uranium, chromates, mercuric chloride, cantharidin, tartrates, etc.

others. For an interesting contribution dealing with the relation of functional tests to pathological diagnosis, the reader is referred to an article by Christian,[72] to which an excellent bibliography is appended. The author of this review very justly concludes that tests of renal function are quite capable of demonstrating the bare fact (the important fact indeed) that the kidneys are diseased but are quite unable to disclose the exact type of pathological lesion of the kidney, which exists in any given case.

While no functional test is capable of disclosing the nature or location of the pathological lesion, it may be perfectly capable of disclosing the inability of the kidney to perform a given function or set of functions, such, for example, as the elimination of water, salt, urea or some foreign substance such as lactose or phthalein. Such an inability on the part of the kidney will indicate a depreciation of its functional power if the test is properly carried out, and this particular information may be important in prognosis and treatment although we shall be unable with certainty to point to the seat of the disturbance in the kidney or identify with exactitude the nature of the underlying pathological lesion.

From a medical standpoint renal function tests are most important in defining the state of kidney activity in the acute and chronic nephritides, orthostatic and other albuminurias, arteriosclerosis, uremia, and myocardial insufficiencies.

In some medical clinics renal function tests are applied as a matter of routine to all these classes of cases. While they do not in themselves make the diagnosis or settle the prognosis it is contended, very properly perhaps, that they will occasionally reveal an unsuspected

[72] Trans. Cong. Amer. Phys. and Surg., 1913, IX, 1.

latent deterioration of kidney function, just as routine blood examinations may reveal an unsuspected leukemia.

Renal functional tests will be of prognostic value because they will often serve to show whether the disease is stationary, progressing or recovering. Of course it hardly needs to be said that renal function tests will always be carried out in the medical clinic in conjunction with the clinical study of the patient. A progressive lowering of the kidney function in chronic nephritis may indicate impending uremia.

The question of the prognostic value of renal functional tests has been made the theme of a very complete and excellent article by Rowntree.[73] According to this investigator the prognostic value of renal functional studies is as great in medical as it is in surgical cases. In acute nephritis, the prognosis depends upon the etiology more than the result of functional tests. In mild chronic nephritis with slight albuminuria and cylindruria, slight hyperpiesis, moderate arteriosclerosis and hypertrophy, the tests may show how great the functional deterioration may be, and the regular repetition of the tests will show whether the disease is progressing or not. In advanced nephritis also, the proper functional tests will disclose the severity of the disease and the imminence of uremia. Cases of clinically well-marked nephritis, even with some uremic signs and a less marked functional derangement, will be more difficult to prognose. Head and brain complications cannot be foreseen by means of renal functional tests. If the renal function remains fair, say 30% of phthalein output (Rowntree) and the blood tests do not show marked cumulative phenomena the prognosis is favorable and vice versa.

In cardiorenal cases it is always difficult to deter-

[73] Trans. Cong. Amer. Physic. and Surg., 1913, IX, 23.

mine clinically just how much the heart and kidney are separately responsible for the conditions. Renal functional tests are of considerable service in showing just how far the kidney is affected. A low phthalein output with cumulative signs in the blood indicate a severe degree of renal involvement. In mere passive congestion these signs are not apt to be found.

Moderately advanced nephritis with slight heart failure may show a fairly good renal function, and, if so, the prognosis depends more upon the response of the heart to treatment than on the kidney. If the phthalein output increases in such cases it is a favorable sign, and if the phthalein output is fairly good in an apparently severe cardiorenal case, the heart may be judged the principal offender in the symptom complex. On the contrary, if the phthalein output is very low and there are signs of cumulation in the blood tests (+urea, +rest nitrogen, low δ*) both kidneys and heart may be regarded as failing and the prognosis is grave.

As a rule in cases of pure myocardial insufficiency the renal function tests give surprisingly good results. When the kidney is passively congested for long periods, however, as a result of heart failure, the functional tests give low results just as they do in severe types of nephritis. The prognosis becomes proportionately lugubrious.

2. *Surgical Application.*—The surgeon is extremely interested in the problems of renal function. With him the question often becomes a very vital one in connection with important and serious operations upon the genitourinary tract. The proper selection of tests is a matter of great importance to the surgeon. Geraghty rightly insists that it is only through familiarity with the reliability, limitations, and significance of findings

* δ, symbol for freezing point of the blood.

of individual tests of renal function, in their relation to various types of disease and various kinds of problems to be solved, that a proper selection can be made.

Certain of the tests or combinations of tests are more suitable to surgical investigations, while other tests or combinations of tests may be of greater value to the internist.

In discussing the utility of renal function tests to the surgeon, we shall draw freely upon Geraghty, since he more than any other investigator has identified himself in his researches with the question of their value and limitations in surgical practice. Some of the tests of renal function are adapted to estimating the total functional capacity of the kidneys at any given time. Other tests are not of great use in this respect but are of considerable importance in determining the relative functional capacity of the organs when applied to the excretion obtained by ureteral catheterization. Finally there are a few tests which are apparently useful in both cases. As an illustration, it is known that the estimation of urea output in a 24-hour specimen is of no great value by itself in judging the total functional capacity of the kidneys, while comparisons of urea elimination in catheterized specimens from both sides may give very important information.

From a purely practical standpoint, as was mentioned under preliminary considerations, all tests for kidney function come under two general heads: 1. Tests to determine how much of a given substance is excreted by the kidney or kidneys, comparing the amount with what is known to be normal. The substance excreted may be one which is normally found in the urine or it may be a substance artificially introduced into the circulation to determine the capacity of the kidneys to eliminate it. 2. The blood may be exam-

ined for substances which are normally passed through the kidneys, with a view of detecting an accumulation of such substances in the body, such accumulation being regarded as an evidence of defective kidney function.

Renal functional tests are especially valuable to the genitourinary surgeon in two types of cases: 1st, Surgical diseases of the kidney secondary to obstructions in the lower urinary tract. 2nd, Unilateral and bilateral surgical diseases of the kidney not associated with obstruction.

Rowntree and Geraghty, in many of their publications concerning the phenolsulphonephthalein test, have called attention to the fact that inasmuch as many cases with obstruction in the lower tract also have hydronephrosis, pyonephrosis, pyelonephritis, or pressure atrophy, an examination of the total function by means of the phthalein test will often show diminished functional activity. In these cases the urinalysis may be misleading, for urea output and total solids secretion may be normal, and yet the kidney function may be so unstable that the shock of a surgical operation, such, for example, as prostatectomy, may be sufficient to inhibit function altogether, and death will result. In just such circumstances they believe a total examination by phthalein will serve to differentiate these cases with severe from those with slight renal involvement.

Experience has shown that cases of obstruction in which the phthalein output is deficient may be so greatly improved by preliminary treatment with proper drainage that the subsequent radical operation may be performed with greatly diminished risk.

According to Geraghty the phthalein test affords the truest index of functional capacity of the kidney for surgical work. The diastase test and urea estimation are, he thinks, of equal value, but are unreliable indices

of functional capacity; but when persistently low values are given by them they may be important from a prognostic point of view. Estimations of blood urea and incoagulable blood nitrogen are extremely important when they are associated with the phthalein test. When all three are positive they are extremely significant.

The functional tests, one and all, can never be said to arbitrarily answer the important question, when to operate and when not to operate, because there are other factors to consider in a surgical case besides renal function. If the phthalein test is very low operation should be postponed until efforts are made by improved drainage (either by suprapubic cystotomy or by catheter) to bring about improvement in the functional capacity of the kidneys.

In the second group of surgical cases above mentioned, namely, unilateral surgical kidney diseases, the relative functional power of the kidneys is a question of vital importance. It is here that ureteral catheterization is of supreme importance, for by this means the excretion from each kidney can be separately obtained.

Many functional tests, such as urea estimations, certain chromoscopic tests, as indigo carmin and methylene blue, cryoscopy, diastase estimation, phloridzin reaction, and experimental polyuria, give information only with respect to the relative functional value of the two kidneys. This is insufficient, since one kidney may be doing a great deal more work than the other, and yet be incapable of doing the work of both, and this is the question of vital importance to the surgeon, requiring a definite answer in each individual case. The phenolsulphonephthalein test has proven of special value here because by it not only is an idea of the total functional capacity, but also a quantitative estimate of the work

done by each kidney obtained.

There are two difficulties, however, which belong to all tests of renal function when carried out with ureteral catheterization; one is inhibition of function produced by the presence of the catheter in the ureter; the other is leakage around the catheter. Special catheters are now used to prevent the latter difficulty. As to the first, the best method of procedure is to test the total functional capacity with phthalein before ureteral catheterization is performed. If the total phthalein output is nearly normal one kidney at least is satisfactorily normal. If, after ureteral catheterization, one kidney is found with an exceedingly low output, which, together with the clinical findings, indicates that this is the diseased organ, it may be safely removed even if the output in the sound side is low, for here the natural inference is that inhibition has produced the deficiency. Inasmuch, however, as inhibition is not necessarily equal on the two sides it will always be necessary to combine the phthalein test with comparison of diastase and particularly urea percentages, on the two sides. The actual procedure recommended by Geraghty is, first, the estimation of total output by phthalein and the estimation of relative function by ureteral catheterization combined with pigment, diastase and urea estimation on the two sides. If the phthalein output is low, cryoscopy of the blood serum and estimation of blood urea may profitably be done.

Geraghty says: "Most of the criticism of functional tests has come from those who have not used them, and are unfamiliar with the nature of the information supplied. It is true that in the vast majority of cases a successful nephrectomy on the diseased side from the standpoint of renal function, can be performed when the urine from the opposite kidney is found apparently

normal on analysis. Unfortunately, however, the problems are not always so simple. Cases of bilateral disease are encountered in which a knowledge of the renal function becomes of absolutely vital importance, and in which every source of information must be called upon before the proper line of procedure can be employed. Again, in certain cases, particularly those of tuberculosis, it may be possible to introduce a catheter only on one side. In such cases one must depend to a great extent upon the information derived from function tests as to the condition of the opposite kidney since the cystoscopic appearance of the ureteral orifice is frequently deceptive. The recognition of hypoplastic and infantile kidney is practically impossible without functional estimation. The infantile kidney is a particularly dangerous type because the urine which is secreted by the kidney is apparently normal in every respect except that of quantity. Functional estimation has proved also of great value in the differentiation between pyelitis and pyelonephritis. In pyelitis the renal function is practically normal, while in pyelonephritis there is diminished function."

SELECTION AND PRACTICABILITY OF RENAL FUNCTION TESTS

In this regard we can do no better than quote the words of Geraghty, spoken at the conference on Renal Function Tests at the ninth triennial meeting of the Congress of American Physicians and Surgeons, 1913 [74]:

"The number of functional tests has become so great that it is impracticable to employ all of them in any individual case; and, even if not impracticable,

[74] See Trans. Cong. Amer. Phys. and Surg., 1913, IX, 45.

nothing would be gained by employing all of these tests. The information furnished by many is of the same character, but more accurately furnished by one test than by others. For example, there is a parallelism between the excretion of the different dye substances; but as phthalein furnishes more accurately all the information obtainable from this group of substances, no advantage attaches to the employment of all.

"For chromocystoscopy alone indigo carmin is unquestionably the test of choice. The estimation of rest nitrogen and blood urea bear about the same significance. Lately we have discarded estimations of residual nitrogen in the blood and are depending entirely upon the blood urea determined by Marshall's method or upon cryoscopy for evidence of cumulative phenomena.

"From a practical standpoint certain tests can be entirely discarded without loss, such as cryoscopy of the urine and electrical conductivity of the urine. Total urea estimations in urine are of doubtful value and diastase determination furnishes only information that is obtainable more accurately and quickly by other means. Certain other tests, such as potassium iodide elimination, can be discarded as furnishing at times unreliable information. We have seen potassium iodide excretion delayed in cases with normal function (proven by subsequent history) and excreted within normal limits in cases of the most severe nephritis. The tests which we consider of the greatest value in the excretory group, based upon actual experience, are: Phthalein, lactose, and chlorides; and of the tests of retention, blood urea, rest nitrogen and cryoscopy. The indications for the specific employments of the individual tests are as follows:

"Chloride estimation in the urine is useful in all forms of nephritis and cardiorenal disease, especially

if œdema is present.

"Lactose is indicated for the detection of slight injury to the kidneys and also in severe nephritis, since its suppression indicates a bad prognosis. It is not particularly helpful in surgical diseases.

"Of the retention tests either blood urea, rest nitrogen or cryoscopy is indicated wherever there is a severe lesion of the kidneys.

"We consider that one of these tests should be used as a routine in conjunction with phthalein wherever functional tests are desirable, particularly if the phthalein function is low.

"Tests in conjunction with ureteral catheterization: in this connection, phthalein, urea in urine, and urinary diastase are most serviceable. The diastase and urea give practically the same information, but only give relative functional values, while phenolsulphonphthalein gives relative and absolute values. The total function should always be estimated by means of phthalein without ureteral catheterization, in order to detect the amount of catheter inhibition, should this exist. Where severe bilateral lesions exist, one of the retention tests should be used.

"As to practicability, the simplest and easiest test is undoubtedly the phthalein test, as it requires the least amount of time and apparatus.

"The lactose test, if quantitative determination is required, necessitates the employment of an expensive polariscope. Furthermore the preparation of the lactose for injection requires attention and consumes time. Its use also requires familiarity with the technic of intravenous injection.

"Diastase requires the daily quantitative preparation of soluble starch, accurately graduated pipettes, a large series of test tubes, a water bath, and one-fiftieth nor-

mal iodine solution. For total estimation it requires 24-hour specimens of urine with preservatives. The time necessary for a simple determination is scarcely warranted by the information obtained. Urea estimations of the urine can be accurately and rapidly done by the Marshall method; and from the standpoint of practicability it leaves little to be desired. It is useful only in conjunction with ureteral catheterization.

"Chloride estimation requires standardized solutions and carefully graduated apparatus when accurately done. It consumes considerable time, and, besides, requires daily collections of the urine with the knowledge of the daily chloride intake.

"All retention tests require, of course, the withdrawal of blood; and cryoscopy of the blood is undoubtedly the simplest, provided that proper apparatus is at hand. It requires careful attention to the details and consumes considerable time.

"Blood urea can be done by either the Folin or the Marshall method, and the total rest nitrogen, preferably by Folin's method; but any of these methods is impracticable for the general practitioner.

"Where only one test can be employed the most value is unquestionably to be obtained from the use of phthalein; and this is particularly so from the standpoint of the surgeon."

Some of the suggestions and conclusions of Mosenthal and Lewis seem especially pertinent. They particularly warn against the phrase, "testing the kidney function as a whole," as it is a well-known fact that the various physiological units of the kidney do not functionate synchronously. Nor do they approve of using a single method for testing the renal function, to the exclusion of all others, as no test constitutes a method

of kidney function. Each test has a significance of its own, and it is only by the use of the various tests that a real insight into the function of the organ is obtained.

They further propose a scale for a more definite nomenclature for the classification of the different degrees of renal function. This scale will probably be changed from time to time, although appearing logical and practical at this time. This proposed grading of impaired renal function should prove a decided boon and should begin to bring order out of chaos. Heretofore, the terms "moderate" or "marked" indicated only what the author had in his mind at that time. Under the proposed classification, the terms "slight," "moderate," "marked," and "maximal" refer to definite mathematical values, something long sought in the medical diagnosis of the kidney conditions.

The same authors have reached several conclusions which seem to be very important, and are as follows:

The relationship between tests for nephritic function and prognosis has been found to be very uncertain. The extrarenal factors, cerebral hemorrhage, myocardial insufficiency, intercurrent infections, etc., have caused a fatal termination so frequently as to put a greater emphasis on the physician's clinical judgment than on the interpretation of tests for renal functions alone.

The level of nonprotein nitrogen and urea nitrogen of the blood must be estimated largely as the resultant of three factors—kidney efficiency, diet and protein destruction. In judging of prognosis, when these substances are high in the blood of nephritics, due regard must be given as to whether their accumulation is brought about by retention alone or through retention coupled with protein destruction. The former offers a comparatively better prognosis than the latter.

TABLE 1.—SCALE OF DEGREE OF IMPAIRMENT OF RENAL FUNCTION AS INDICATED BY THE TESTS EMPLOYED

Degree of Impairment of Renal Function		Phenol-sulphone-phthalein, per cent.	Non-protein N of the Blood, Mg. per 100 c.c.	Urea N of the Blood, Mg. per 100 c.c.	Ambard's Coefficient of Urea Excretion	Test Meal for Renal Function					
						Night Urine		Variations in Sp. G. When the Highest Sp. G. is:			
						C.c.	Sp. G.	18	17–15	14 and 13	12—
Normal.....	0	60+	30—	15—	0.090—	400—	18+	9+	6+		
Slight.......	+	59–40	31–45	16–27	0.091–0.115	401–600	16 and 17	8–5	5 and 4	6+	
Moderate...	++	39–25	46–65	28–44	0.116–0.220	601+	15—	4—	3—	4 and 5	6+
Marked.....	+++	24–11	66–90	45–64	0.221–0.350	....	....	....	....	3—	5—
Maximal....	++++	10– 0	91+	65+	0.351+	....	....	....			

The coefficient of Ambard is a better method of determining the ability of the kidney to excrete urea than the level of this substance in the blood.

The progress of renal disease is probably followed most minutely by means of the phenolsulphonphthalein excretion and Ambard's coefficient, as these tests furnish figures in which even small variations are of significance.

The test meal for renal function, aside from the uric acid estimation in the blood, gives the earliest indication of diminished kidney efficiency. It likewise reaches the maximum degree of impairment before the others.

The value for early diagnosis of an increase of uric acid in the blood, and for prognosis, of an increase of creatinin in the blood, might well be emphasized.

Each test for renal function covers only a limited range of the kidney's activities. It is, therefore, a mistake to speak of any one test as measuring renal function as a whole. The aim should be to develop a proper interpretation of the old tests and the easily applied new ones, in order to obtain a true guide to treatment of diseases of the kidney.

See Mosenthal and Lewis: J. A. M. A., 1916, lxvii, 933-938.

CHAPTER III

TESTS OF PANCREATIC FUNCTION

GENERAL CONSIDERATIONS

As Stadmüller [1] rightly says, "The recognition in the living subject of pathological conditions of the pancreas belongs, without doubt, to the more difficult problems of the diagnostic technic of the present day."

Aser [2] also said years ago that there is no organ in the body in which such a disparity exists between its known physiological importance and our capacity to clinically estimate its functioning power.

In other words, the enormous importance of the pancreas as a digestive organ and as a gland of internal secretion is granted, thanks to the work of Von Mering, Minkowski, Pawlow, Boldyreff, and many others. Our ability to estimate the functioning power, the physiological capacity, the anatomical condition of the organ in a given case is limited.

The importance of the pancreas as a digestive gland is very great. It is the only gland which furnishes an enzyme for each class of foodstuffs.

The activated proteolytic ferment trypsin breaks up protein into simpler structures (amino acids) than the gastric juice. Amylopsin or pancreatic diastase converts starch into sugar. Steapsin or pancreatic lipase

[1] Archiv. of Diag., 1911, IV, 20.
[2] Nothnagel's Spec. Path. und Therap., Wien, 1898, I.

splits neutral fats into fatty acids and glycerine. Glaessner has estimated that the pancreas pours into the duodenum through the ampoule of Vater perhaps a pint or more of its mixed secretion per day.

But beyond this great act, which a priori would appear to be more than sufficient for a single organ, the pancreas elaborates a mysterious secretion which presides in an equally mysterious way over the sugar metabolism of the body.

Von Mering and Minkowski in 1889 discovered that typical diabetes follows total extirpation of the pancreas in dogs. This fact, which has received an immense amount of experimental corroboration, still stands out as an epoch-making discovery in the history of pancreatic physiology amidst much that is obscure and uncertain.

It is pretty generally agreed that the internal secretion of the pancreas is elaborated in the islands of Langerhans, closely crowded groups of polygonal cells without excretory duct of any kind scattered in the stroma of the gland between the external secreting acini and surrounded by a profusely developed net of blood vessels.

Besides controlling the carbohydrate intermediate metabolism of the body the internal secretion of the pancreas is claimed by Loewi and some others to exert an inhibitory effect upon the sympathetic.

From the standpoint of the clinician it is an exceedingly difficult matter to determine the existence of pancreatic disease, and to ascertain its nature. All the customary methods of examination are, of course, employed in such a search. Inspection, palpation, blood examination, and a careful study of the semiology are made use of. The subjects of pancreatic semiology, pain, tenderness, tumor, pressure, etc., and pancre-

atic exploration are extremely important aids and indispensable adjuncts in pancreatic diagnosis. They do not come, however, under the functional investigation of pancreatic activity, and will, therefore, be necessarily left undiscussed in this place.[3]

In considering the question of studying the functional capacity of the pancreas, two possibilities become immediately apparent. (A) Functional investigation may be made of the pancreas regarded as an organ of external or digestive secretion, and (B) the functional examination may relate to an inquiry concerning the internal secretory activity of the organ, its power, in other words, of adequately performing its endocrinous or metabolic function.

I. TESTS FOR PANCREATIC FUNCTION, WHICH CONCERN THE EXTERNAL OR DIGESTIVE ACTIVITY OF THE ORGAN

These may be divided into four subdivisions, as follows:

1. Proteid digestion tests.
2. Fat digestion tests.
3. Starch digestion tests.
4. Demonstration of pancreatic ferments in secretions and excretions.

The proteid digestion tests are: Demonstration of waste muscle fibre in the stools, the so-called azotorrhœa or creatorrhœa. Schmidt's test for digestion of nuclei. Sahli's glutoid capsule test.

The fat digestion tests are: Demonstration of excess fat in the stools, so-called steatorrhœa. Identification of split fat in the stools. Winternitz Diagnosticum.

[3] Consult Opie's Text Book, Diseases of the Pancreas.

The starch digestion test consists of identification of undigested starch in the stools.

Besides the above tests there have been devised special qualitative and quantitative methods of identifying the various pancreatic ferments themselves (trypsin, amylase and lipase) in the stools, urine and gastric contents. It is somewhat difficult, therefore, to separate the operations of routine urinalysis and coproanalysis intended to show the presence or absence of pancreatic enzymes, from certain special tests which have been devised with the express purpose of testing pancreatic function. For example, Schmidt's test for nuclear digestion, and Sahli's glutoid capsule test might, upon superficial consideration, be considered as special tests for pancreatic function, but in reality they are both merely special methods of demonstrating the presence or absence in the intestine of pancreatic enzymes.

However, the fact that in the performance of these tests something particular is done in the way of special preparation with a certain definite end in view, would, in all likelihood, insure their inclusion in any scheme or list of functional pancreatic tests.

All the other tests mentioned in the above synopsis are, in reality, only special methods of urinalysis or fecal examination.

The same difficulty of classification becomes apparent when we come to consider the tests of internal secretion. The Cammidge test, as it is called, is but a specially technical urinalysis.

The Loewi test for pupillary reaction to adrenalin and the test for alimentary glycosuria might be said to answer all the requirements of special functional tests, but if our knowledge of pancreatic function were limited to these experimental inquiries alone, it would be

meagre indeed.

From all these considerations it will be apparent that any attempt to give a description of methods of studying pancreatic function must include all of the abovementioned methods, although a very strict interpretation might relegate some or many of them to the domain which is usually covered by books dealing with chemical or microscopical laboratory methods.

II. TESTS FOR PANCREATIC FUNCTION WHICH CONCERN THE INTERNAL OR METABOLIC FUNCTION OF THE ORGAN

There are three tests which belong to this class.

1. The Cammidge reaction.
2. Loewi's pupillary reaction.
3. Provocative alimentary glycosuria.

These will be described in their proper place.

1. Proteid Digestion Tests. Estimation of Undigested Protein in the Stools as a Means of Determining Pancreatic Hypofunction

The patient is placed for three days on Schmidt's test diet. This diet is as follows: 1.5 liters of milk, 100 gms. of zwieback, 2 eggs, 50 gms. of butter, 125 gms. of beef, 190 gms. of potatoes, and gruel made from 80 gms. of oatmeal. In this diet are contained 102 gms. of albumen, 111 gms. of fat, 191 gms. of carbohydrates; making a total of 2234 calories of energy.

According to Schmidt the diet is distributed through the day as follows: Morning, .5 liter of milk (if milk does not agree .5 liter of cocoa made from 20 gms. of cocoa powder, 10 gms. sugar, 400 gms. water and 100 gms. milk are substituted), 50 gms. zwieback; noon, 125

gms. chopped beef (raw weight) broiled rare, with 20 gms. of butter (the interior of the meat must be raw), 250 gms. of potato broth made of 190 gms. of mashed potatoes mixed with 100 c.c. of milk and 10 gms. of butter; afternoon, same as morning; evening, same as forenoon.

It would be well if investigators should agree to use this well-known standard diet so that comparisons of results could be made.

Pratt,[4] while using the diet, recommends that the whole quantity be given in three meals instead of five, conforming thus to American custom.

The Schmidt diet, containing as it does a good amount of all three varieties of foodstuffs, is adapted to all tests which involve an examination of the feces.

The presence of azotorrhœa or defective proteid digestion due to deficiency of trypsin zymase, is determined by examining the feces microscopically after the test diet.

Meat consists of connective tissue and muscle fiber. Connective tissue is digested promptly by the gastric juice, the muscle fiber by trypsin. Large quantities of striated muscle fiber in the feces may indicate defective pancreatic activity.

The bulkiness of the stools should also be noted. Oser, Musser and others have claimed that there is no single symptom of pancreatic disease of greater significance than bulkiness of the stools. The dried weight of the stools may be rather readily ascertained. A ventilating hood and scales are all that are necessary. If the pancreatic juice is not appearing in the bowel the dried stools will weigh more and the stools themselves will be more than ordinarily voluminous.

In six healthy individuals on Schmidt's diet Pratt

[4] Amer. Jour. Med. Sc., 1912, CXLIII, 313.

found the average weight of the dried feces to be 54.3 gms. The maximum was 62 and the minimum 45 gms.

Schmidt's Cell Nuclei Test for Pancreatic Sufficiency.[5]—According to Schmidt, the nuclei of animal cells is digested only by the pancreatic enzymes. Considerable has been written pro and con concerning this test and, although it does not appear to be extensively used, there is no doubt that it is not devoid of a certain value.

Technic.—Raw beef somewhat fibrous is cut into cubes of .5 c.cm. These are hardened in alcohol and placed in small bags of coarse silk gauze. Before using they must be soaked several hours in water. The bags are recovered in the stools, hardened, paraffined, cut and stained, and the sections mounted and examined microscopically for nuclei. If the nuclei are not digested, they will take the stain and become visible. Thymus gland has been substituted for the meat by Einhorn.

Kashiwado, one of Schmidt's pupils, has altered the original test by giving a powder consisting of equal parts of stained thymus nuclei and lycopodium. The mixture is given in two capsules, each containing .25 gm., at the evening meal. The stool is examined for stained nuclei.

In performing the Schmidt test the sac must be recovered in the feces before the expiration of 30 hours at the latest, otherwise a longer sojourn in the bowel will enable the bacterial enzymes to dissolve the nuclei.

Sahli's[6] *Glutoid Capsule Test of Pancreatic Function.*—The glutoid capsules used in this test are made of gelatin and hardened in formalin. This is intended

[5] Verhandl. d. Kong. f. inn. Med., 1904, XXI, 335.

[6] Deutsch. med. Wchnschr., 1897, XXIII, 6; also Deutsch. Archiv f. klin. Med., 1898, LXI, 475.

to make them resist digestion by the gastric juice, to permit of their entering the bowel, there to be softened and disintegrated by the pancreatic juice if it be present in sufficient amount. It is the trypsin zymase which affects this solution.

The capsules may be filled with sodium iodide, iodoform or salol. The last is usually employed. Salol is split up by the pancreatic juice into salicylic acid and phenol. The former is eliminated by the kidney and escapes in the urine as salicyluric acid, which is easily recognized in the excretion by the violet color produced by adding to the urine a few drops of a solution of ferric chloride.

Normally the reaction is obtained in five hours. Sahli did not disclose the precise manner of preparing the capsules and this, according to Pratt, has hindered the generalization of the test which is so extremely simple.

The capsules are made and sold by Hansmann of St. Gallen, Switzerland.

Sailer[7] says that a satisfactory capsule may be prepared by placing ordinary gelatin capsules in pure formalin for three minutes.

This test is practically the same as that introduced by Sahli for estimating gastric motility. Its extreme facility of execution would make it an excellent test if there were unanimity in the findings. Unfortunately this is not the case and at the present time no one would pretend to depend upon its results alone, though it seems undoubtedly true that in the absence of pyloric spasm or stenosis, the capsule test of Sahli is worth considering from a corroborative point of view.

[7] Amer. Jour. Med. Sc., 1910, CXL, 330.

2. Fat Digestion Tests. Demonstration of Excess of Fat in the Stools (Steatorrhœa) and Diminution of Split Fats in the Stools as a Means of Estimating Pancreatic Insufficiency

Here the insufficiency of pancreatic function, if it exists, is reflected upon the fat-splitting activity of the external secretion and shows itself by phenomena which are due to diminution or absence of the lipasic enzyme of the juice.

Steatorrhœa, or increased fat in the stools, is known to be common in pancreatic disease. The light color of the stools is an important macroscopic feature. They may be almost white. The stools are often rancid in smell and bulky. Since the stools in icterus may also be fatty it may be necessary to test for bile in order to make sure that the pale color is not due to hydrobilirubin. When fat crystals are present in excess, the stools have a metallic lustre like aluminum. A white stool, fluid when passed and becoming solid on cooling, is fatty and is said to be quite characteristic of defective pancreatic secretion. The most important function of pancreatic lipase is to split up the neutral fats into fatty acids and glycerine. The free fatty acids combine with the alkalies of the pancreatic juice to form readily assimilable soaps.

If the functional activity of the pancreas is deficient, one important consequence will be a considerable diminution in the amount of split fats, and hence of soaps. The fat will remain unsplit, therefore unassimilable and consequently unabsorbed.

Normally from 7 to 11% of the fat ingested in the food escapes action by the pancreatic juice and is passed in the stools. If bile is absent from the intestine because of occlusion of the duct, there will be incomplete

emulsification of the fats and consequent reduction of pancreatic effects. Under such circumstances as much as 45% of the fats ingested may escape in the stools. If, in a case of icterus, the loss of fat in the stools is less than 60% the pancreas is probably not implicated. If the fat loss exceeds 60% it points to pancreatic disease.

Under the microscope, fat appears in the stools as droplets, needle crystals, or as structureless plates or flakes. Unfortunately the quantitative estimation of fat in the stools is not a simple matter. The stools must first be dried on a water bath; the neutral fats and fatty acids are extracted in a Soxhlet apparatus with ether, from a known quantity of dried stool; the residue is treated with diluted HCl to convert soaps into fatty acids and these are extracted in the same manner. The amount of free acid in the first extract is calculated with titration with alkali.

Like the Cammidge reaction or test (*v.i.*) the quantitative estimation of fat in the feces is not to be regarded as a clinical procedure likely to be carried out by the physician. The method requires some apparatus and more chemical experience than the clinician is likely to possess and, besides, is time consuming. Of course, in many hospitals, the examination can readily be made. It is not considered advisable to give the details of the method for fat extraction and quantitative estimation, the skeleton of which is above outlined, especially as the details may readily be obtained from texts on chemical diagnosis. For convenience two references may be given—Sahli [8] and Wood.[9]

Winternitz's Test of Pancreatic Fat-splitting Function. Winternitz Diagnostikum. The Sajodin Test.—This test is founded upon the fat-splitting power of

[8] Diagnostic Methods, 1905, p. 446.
[9] Chemical and Microscopical Diagnosis, 1909, p. 334.

steapsin to set free iodine from iodine-containing fats. Winternitz was the first to apply the artificially iodized fats to the investigation of the fat-splitting power of the pancreatic juice. The first substance used was iodipin, but it was found that the iodine present in this substance is so firmly bound that even normal pancreatic juice may not split it. He finally selected monoiodobehenate of calcium or sajodin as the most suitable substance.

Sajodin is a thin, oily liquid containing 25% of iodine. If 3 c.c. of this substance are administered by mouth to a fasting individual and the urine examined for iodine 3 to 5 hours later the reaction will be negative. If the same substance in the same amount is given with a meal, the iodine reaction will be present. In the first instance no pancreatic secretion has been stimulated to appear in the duodenum and consequently the sajodin is not split. In the second instance the opposite prevails.

It was further found that in cases of icterus no splitting of sajodin takes place because of the absence of bile salts in the intestine, which are necessary to actuate the fat-splitting ferment and to stimulate absorption.

Several investigators have investigated the Winternitz test and the question has been made the subject of a thesis by Stegman.[10] He concludes that the failure to find iodine in the urine 3-5 hours after the ingestion of 3-5 c.c. of sajodin with a meal, is indicative of lipolytic pancreatic insufficiency in most cases, and that, in combination with other well-known tests, the method of Winternitz may be regarded as of considerable corroborative value.

[10] Ueber eine neue Methode der Pankreasfunktions prufung Winternitz Diagnostikum. Dissertation Otto Stegman, 1911.

In a recent inaugural dissertation upon the Winternitz sajodin test by Syring,[11] this author finds that without exception in normal cases iodine appears in the urine in 3-5 hours after the ingestion, with a meal, of 5 c.c. of calcium monoiodobehenate (sajodin).

The question as to its real value in determining the existence of pancreatic insufficiency can only be settled by further investigations.

3. Starch Digestion Test. Identification of Undigested Starches in the Stools as an Evidence of Pancreatic Insufficiency

The presence of starch in the stools is generally understood to be of little value in this connection. Normally scattered granules of undigested starch are to be found and can be easily identified under the microscope, when stained with iodine solution (Lugol's), which colors them blue. Any great excess, however, may fairly awaken suspicion, especially if there is persistent diarrhœa and the condition tends to be permanent or long continued. Under these circumstances it is legitimate to conclude that there is pancreatic insufficiency.

4. Identification of Various Ferments. Tests Where Examination Is Made for the Pancreatic Ferments Themselves in the Excreta. The Meaning and Interpretation of These Results in Relation to Estimating the Pancreatic Function

The different pancreatic ferments can be demonstrated by proper methods, in the stomach (after an oil meal), in the urine, and feces.

Einhorn, Gross, and others have devised special ap-

[11] Ueber die Funktionsprufung des Pankreas. Leipzig, 1913.

paratus for obtaining the pancreatic juice directly from the duodenum. Einhorn's duodenal tube is used considerably in this country, but more particularly for therapeutic purposes.

One objection which urges against all tests for trypsin in the stools is that erepsin, a ferment coming from the mucous membrane of the small intestine, may digest albumen, etc., even in the absence of trypsin. The objection cannot be urged against the gastric estimations, and, in fact, seems to be exaggerated even when applied to fecal analysis methods.

Demonstration of Trypsin in the Stools.—Two methods are chiefly used; the Serum Plate method and the Casein method.

The Serum Plate Method of Müller and Schlecht.[12]—Trypsin acts upon the surface of serum agar plates.

Method. One drop of the stool obtained by a laxative (calomel or phenolphthalein) is placed upon a Löffler serum agar plate and kept at a temperature of 55° to 60° C. for 6-12 hours in an incubator.

If trypsin is found in the stool there will appear a depression or hole in the serum due to digestion by the enzyme.

Ordinary diphtheria culture tubes have been suggested by Stadmüller as being sufficient for the purpose of the test.

This test is simple and seems to be rather highly regarded (Brugsch, Hirshberg, etc.). Other practical methods of demonstrating trypsin in the feces have been devised. Arthur and Hubert add a 2% solution of sodium fluoride to the stools, also fibrin, and incubate at 40° for 24 hours. Crystals of tyrosin are formed if trypsin be present. Abderhalden's technique has also been employed, using glycyl-tyrosin. The Müller-

[12] Münch. med. Wchnschr., 1908, LV, 225.

Schlecht method is sufficient, however, and much more simple.

For a quantitative variation of the serum agar plate method of stool examination for trypsin, put in one part of the plate undiluted feces and in orderly sequence in other portions of the plate use dilutions of the feces 1:10, 1:20, 1:100, 1:200, and note which still forms indentation. If the stools are fatty the fat should be extracted with ether.

The Casein Method of Demonstrating Trypsin in the Stools.—Casein in alkaline solution is precipitated by dilute acetic acid. If casein is digested by trypsin it will no longer give the precipitation reaction with dilute acetic acid. This is the foundation of a test devised by Gross.[13]

In Gross' method the feces are mixed with an alkaline solution of casein—.1% casein, .1% sodium carbonate. Various quantities of the filtered feces are added to the casein solution, incubated for an hour and tested with dilute acetic acid (*v.i.*).

Mette's tubes are also sometimes used in testing for the presence of trypsin. They consist of glass tubes about 1 or 2 mm. in diameter, containing coagulated egg albumen. These are suspended in the dilute feces or other solution to be tested for a fixed time and the amount of albumen digested off, measured. The strength of the ferment will be proportional to the square of the length digested.

Technic of Gross' Quantitative Test for Trypsin.—Prepare a .1% solution of casein by adding 1 gm. of pure casein (Merck) and 1 gm. of sodium carbonate to 1000 c.c. of chloroform water. Place in a flask and

[13] Arch. f. Exper. Path. und Pharm., 1907, LVIII, 157; also Deutsch. med. Wchnschr., 1909, XXXV, 1706.

allow to stand for 24 hours, after which the solution is shaken vigorously.

Five gms. of feces are placed in a mortar. Add 45 c.c. of 1% solution of sodium carbonate. Titrate thoroughly and filter. The first cloudy portion of filtrate is discarded. The second is used.

To each of six reagent glasses marked for identification add 10 c.c. of the casein solution. With graduated pipette add respectively 1 gm., .5 gm., .25 gm., .2 gm. and .1 gm. of filtered feces to specimen and mix thoroughly. Place all in incubator, adding to each 3 drops of 1% acetic acid. Specimens in which the casein are digested (presence of trypsin) will remain clear; others are cloudy.

In normal stools, glasses 1 to 3 are clear, 4 to 6 cloudy. A trypsin unit equals the amount of feces which digests 10 c.c. of starch casein solution. If .33 gm. feces which is diluted tenfold digests 10 c.c. of casein solution, there are 30 trypsin units, which is normal. In clinical work, disease of the pancreas may be suspected when no trypsin or, at most, 10 units are found in examination of the feces.

Demonstration of Trypsin in the Stomach Contents. Method of Boldyreff-Volhard.[14]—Boldyreff noted in 1904 in Pawlow's Institute that feeding olive oil to dogs caused regurgitation of duodenal contents into the stomach. Volhard [15] applied the principle to the clinic.

A breakfast of 200 c.c. of olive oil is given by stomach tube or 250 c.c. of cream may be substituted, the latter being swallowed. Half a teaspoonful of magnesia usta are given just prior to the meal and is repeated

[14] Centrbl. f. Physiol., 1904, XVIII, 457; also Zentbl. f. Phys. und Path. d. Stoffw., 1909, III, 209.
[15] Münch. med. Wchnschr., 1907, LIV, 403.

twenty minutes afterward. This is to prevent acidification. At the end of 45 to 60 minutes, the stomach contents are removed by tube. Usually a liquid is obtained which tends to separate into two layers, the lower one containing the duodenal juice.

The presence of trypsin can be demonstrated by the Gross casein test described above or by that of Arthur Hubert,[16] previously mentioned, the details of which follow:

Fresh fibrin obtained from horse blood by whipping and washing the coagulum is covered with 2% solution of sodium fluoride and kept for 24 hours at 40° C., then filtered.

The fluid to be examined is diluted with equal volume of 2% sodium fluoride solution, and one volume of this dilution is added to two or three volumes of the fibrin solution and digested at 40° for some hours. Crystals or crusts of tyrosin form on the wall of the vessel.

According to Sahli trypsin can qualitatively be most easily demonstrated by digesting in alkaline fluid at incubator temperature a flake of fibrin stained with magenta red. The fibrin becomes digested and dissolved and the fluid is colored red.

Stadmüller mentions a simple qualitative test devised by Von Oefele. A few drops of Fehling's alkali solution with a few drops of a 1-1000 solution of casein are added to a .07% copper sulphate solution and .1% sodium carbonate. The mixture is incubated at 55°. To 5 c.c. of this, in a warm test tube, are added five drops of the fluid (intestinal juice) to be tested, the whole being shaken. If trypsin is present the solution which at first is blue or green if bile is present becomes red-violet or rose color.

[16] Archiv. de Physiol., 1894, 622.

Estimation of Diastatic and Lipolytic Ferment in the Feces as a Measure of Pancreatic Function.—The results obtained by a study of the diastase content of the stool should, provided all controllable factors in performing the test are standardized, give valuable information concerning the functional integrity of the pancreas. The physiological basis upon which the test is founded is, that practically the whole amount of diastatic ferment found in the feces is of pancreatic origin, i.e., provided certain possible sources of error are understood and obviated (Wohlgemuth).

Several satisfactory methods for estimating diastase in the feces have been devised, chief of which are those of Wohlgemuth [17] chiefly used in Germany; that of Durand,[18] chiefly used in France and England, and that of Brown,[19] chiefly used in the United States. The methods of Durand and Brown will be described.

Durand's Method of Estimating Diastase in the Feces.—One c.c. of the total diluted feces is added to 50 c.c. of starch solution (1% starch and 2% decinormal HCl). The tubes are incubated for half an hour at 39.5° C. and digestion is then stopped with three drops of strong soda solution. The sugar formed is estimated quantitatively with Fehling's solution.

Ten c.c. Fehling's solution is reduced by .0124 gm. of sugar. If x be the number of c.c. of the incubated mixture used to reduce the Fehling's solution, the amount of sugar present in the 51 c.c. of the mixture will be $\frac{.0124 \times 51}{x}$ or, in the whole amount of the

[17] Biochem. Zeitsch., 1908, IX, I; also ibid., 1909, XXX, 432.
[18] Archiv. des Mal. d. Appar. Digest., 1911, V, 76.
[19] Johns Hopkins Hosp. Bull., 1914, XXXV, 200.

feces (obtained as below), $\frac{.0124 \times 51 \times 20{,}000.}{x}$ This figure is multiplied by two to give units of grams of sugar formed in the hour. The normal limits are 1500-2000 units.

The feces for the test are obtained as follows: The patient is well purged 12 hours after his last meal. He is then given ¾ liter of milk and 45 minutes later 50 grams sodium sulphate in water, and one-half hour after this a glass of vichy. The feces of the next 3½ hours are passed into a vessel containing ice. They are then diluted with water to 20 liters and tested as above.

Technic of Brown's Test.—The patient is given a high enema the night before. The evening meal should be very light. At 7 A. M. the next morning 750 c.c. of milk are given. At 7:30 and again at 8:30 A. M. ½ ounce of Epsom salts are taken. At 8:30 a glass of water containing ¼ teaspoonful of sodium bicarbonate is swallowed.

All stools up to 2 P. M. are saved in a vessel containing 2 ounces of toluol which is kept in a cool room or on ice. If less than 400 c.c. of stool are obtained an enema of a pint of water is given. The average quantity collected up to 2 P. M. will be from 400 to 1100 c.c. usually.

The stool should be examined as soon as possible after 2 P. M. Dilute the amount up to 3000 c.c. with normal salt solution, stir the whole amount until absolutely homogeneous. Centrifugalize a portion for 5 minutes and use the supernatant fairly clear fluid for testing.

Diminishing amounts of the fluid are put into a series of tubes, 1.8 c.c. in the first, 1.6 c.c. in the second, 1.4 c.c. in the third, 1.2 c.c. in the fourth, 1 c.c. in the

fifth, .8 c.c. in the sixth, .6 c.c. in the seventh, .4 c.c. in the eighth, .2 c.c. in the ninth, .1 c.c. in the tenth, .05 c.c. in the eleventh and .025 c.c. in the twelfth. Bring the fluid in each of the tubes up to 2 c.c. with normal salt solution. If the test shows a negative reading in the first tube or if a low reading is expected, a supplementary set of tubes is prepared containing respectively, 2 c.c, 3 c.c., 4 c.c., and 5 c.c. centrifugalized mixture.

In each of the tubes is added 2 c.c. of 1% solution of soluble starch (Kahlbaum) and the tubes are incubated at 38° C. in water bath ½ hour, then cooled by adding a little tap water and by holding them under the cool tap. They are then quickly tested with a few drops of one tenth normal iodine solution. The limit is held to be that tube before the one in which the first definite blue color appears.

Demonstration of Lipolytic Ferment.—Two simple tests for the presence of lipolytic ferment may be mentioned. These are the Grutzner-Gamgee [20] method and the von Oefele [21] method.

The first is as follows: An emulsion of ten parts of oil, five parts of gum and thirty-five parts of water is prepared. A neutral solution of litmus is made up which in test tubes of 12 mm. diameter appears violet against white paper. Ten c.c. of litmus solution and 5 drops of the emulsion are placed in several of these tubes and increasing quantities, 2, 4, 8, 16, 32 drops, of the fluid to be tested are added to the successive test tubes. These are put in a water bath at 37° C., and after a short time the tubes are compared. If any fat-splitting ferment is present the color of the fluid will have turned redder the larger the amount of solution added.

[20] Quoted by Stadmüller, *loc. cit.*
[21] From Sahli's Diagnostic Methods, 421.

Von Oefele's method of demonstrating steapsin is as follows: sweet butter is melted and the resulting clear fat mixed with an equal proportion of a 1% aqueous solution of potassium carbonate and some phenolphthalein, and then titrated with a soda solution until there is a red tint. This liquid is heated in the incubator to 55° C. and 5 c.c. of it well shaken in a warm test tube with 5 drops of intestinal juice. In the presence of a normal amount of steapsin the red tint will disappear in from 2 to 5 minutes. According to the rapidity of the discoloration the quantity of actual steapsin can be estimated.

II. TESTS FOR PANCREATIC FUNCTION WHICH CONCERN THE INTERNAL OR METABOLIC FUNCTION OF THE ORGAN

All the functional tests of pancreatic activity which have been described have related to the external secretion with its enzymes, which is poured out through the pancreatic duct and the ampoule of Vater into the small intestine.

There is, however, another phase to the question of functional deficiency of the pancreas and this relates to its internal secretion, which in some mysterious or quite unknown manner presides over the mobilization and destruction of sugar in the body. When the internal secreting function of the pancreas is lowered, the power of assimilation of carbohydrates is reduced and when a certain limit is reached a hyperglycemia results which tends at a certain point to manifest itself by the elimination of sugar in the urine, glycosuria, diabetes.

There are three functional tests which concern particularly the internal function of the pancreas. These are:

1. The Cammidge Reaction.
2. Loewi's Pupillary Reaction.
3. Provocative Alimentary Glycosuria.

1. The Cammidge Pancreatic Reaction

There is still much dispute as to just what position the reaction holds in the clinical diagnosis of pancreatic function.

The original Cammidge reaction consisted of two parts or analyses which were known as A and B tests. It was held that the presence of a pancreatic lesion and even its nature could be determined by these tests. The original theory upon which the test was based was about as follows. If there is a real pancreatic lesion, the pancreatic juice will escape into the parenchyma of the organ and lead to fat necrosis with splitting of neutral fat into fatty acids and glycerine.

The fatty acids will remain in the necrotic areas and the glycerine will be absorbed into the blood and excreted by the urine. The Cammidge test was devised to demonstrate the presence of glycerine in the urine by the presence of glycerosazone crystals.

The original theory was subsequently modified and the two original tests were abandoned and replaced by one process known as the C test. The present theory of the Cammidge test is this. The crystals produced in the test when positive, result from the presence in the urine of a sugar complex which upon hydrolysis with HCl yields a substance giving a pentose reaction. The crystals of a positive reaction are believed to be pentosazone.

The pancreas contains four or five times as much pentose as any other organ in the body and consequently when any disintegration of pancreatic tissue

takes place as a result of disturbance or disease of the organ, crystals of pentosazone, the Cammidge crystals will be demonstrated in the urine. Neither a mere blocking of the pancreatic secretions nor a pure fibrosis of the organ will produce a positive reaction. It is usually held as Cammidge himself believes that a positive reaction is evidence of active degeneration such as occurs in acute or chronic pancreatitis. A negative reaction contraindicates active degeneration but does not exclude old pancreatitis nor malignant disease of the pancreas. In fact, in 75% of malignant cases the reaction is negative. But the test is not always positive even in pancreatitis and a negative test does not definitely exclude pancreatitis. The results must always be taken in conjunction with clinical, urinary and fecal findings. The test is not considered generally by pathologists or clinicians as having great practical value in diagnosis. But when positive it constitutes an interesting abnormality which seems to be connected in some rather cryptic or obscure manner with disturbances of the pancreatic function.

Technic of the Cammidge Test.—Filter a portion of a 24-hour specimen of urine.

Test for Albumen.—If albumen is present in amount more than a trace, measure out 50 c.c. of filtrate and add a few drops of acetic acid, boil, cool, filter and make up to 50 c.c.

Test for Sugar.—Either Fehling's or Nylander's test is performed. The result must be absolutely negative. If there is any reduction on standing about 50 c.c. of the albumen free urine must be mixed with yeast fermented for 12 to 24 hours and filtered.

Stage I. Measure 20 c.c. of the clear albumen and sugar free filtrate into a small flask with an inverted filter funnel placed in its mouth as a condenser.

Add 1 c.c. of strong HCl. Boil on sand bath for 10 minutes from commencement of ebullition. The boiling should not be too vigorous and the flame should be turned low for the greater part of the time.

Stage II. Cool under the tap. Make up contents to 20 c.c. with distilled water. Slowly add 4 gms. of lead carbonate; shake gently at first and more thoroughly later. Stand, and shake occasionally until no more gas comes off. Filter through a paper moistened with distilled water.

Stage III. Add 4 gms. of powdered tribasic acetate. Shake thoroughly for some minutes and allow to stand. Filter through a moistened filter paper.

Stage IV. To the clear and almost colorless filtrate add 2 gms. of powdered sodium sulphate, shake thoroughly for several minutes. Bring slowly up to the boiling point on a sand bath, shaking from time to time. The excess of lead is removed at this stage and it is important that the shaking and heating should be done carefully.

Stage V. Cool under the tap and filter. Measure 10 c.c. of clear filtrate. Make up to 18 c.c. with distilled water. Add 8 gms. of phenyl-hydrazine hydrochlorate, 2 gms. powdered sodium acetate and 1 c.c. of 50% acetic acid.

Boil in a flask with a funnel condenser on the sand bath for 10 minutes from the commencement of ebullition. Do not boil too vigorously. Filter hot through a filter paper moistened with boiling distilled water into a 15 c.c. measure. Should the filtrate fail to reach the 15 c.c. mark make up to 15 c.c. with hot distilled water. Stand for from 4 to 5 hours or longer at room temperature or in ice chest.

Examine the filtrate for the appearance solubility and amount of crystal formation.

The typical crystals examined under the microscope are of the osazone type and more circular and tuft-like than glucosazone crystals. Run under the cover slip 33% H_2SO_4; the crystals should dissolve in 10-15 seconds. The crystals have to be distinguished from the coarse yellow needles which may be deposited if the excess of lead was not removed in *Stage IV*. In a strongly positive reaction the deposit of crystals may occupy half the bulk of the filtrate. In a completely negative reaction the filtrate remains clear.

2. *Loewi's Pupillary Symptom or Test of Pancreatic Insufficiency*

In 1908 Loewi [22] made the observation that after removal of the pancreas in certain animals, the instillation of adrenalin into the eye will cause dilatation of the pupil. Ordinarily the instillation of adrenalin into the eye does not cause dilatation altho intravenous injection will do so. Loewi attributed the mydriasis following instillation to increased excitability of the sympathetic system brought about by the removal of the inhibitory effect of the pancreatic internal secretion.

From this fact it was thought that a mydriasis in man following local instillation of adrenalin would indicate pancreatic internal insufficiency, provided hyperthyroidism or Graves' disease did not exist.

According to Sladden [23] this test has given interesting and encouraging results and should not be dismissed with the comparatively scant attention it has received lately.

The technic of the test is extremely simple since it consists merely of dropping into the conjunctional sac

[22] Archiv. of Exper. Path. and Pharm., 1908, LIX, 83.
[23] Quart. Jour. of Med., 1913-14, VII, 455.

a few drops of a 1:1000 solution of adrenalin and observing the effects upon the pupil.

3. Spontaneous and Provocative Alimentary Glycosuria in Their Relation to Pancreatic Function

The intimacy of relationship between the pancreas and control of the carbohydrate metabolism is close and undisputed, but what particular cells of the pancreas are concerned or just what the mechanism of the control may be is very imperfectly understood. Glycosuria is usually present in many of the more serious pancreatic diseases, but glycosuria is by no means an infallible index of either the extent or the nature of the pathological processes. In many cases of pancreatic disease, however, glycosuria does not appear (e.g. many cases of chronic pancreatitis, carcinoma, etc.). According to Cammidge the presence of glycosuria means only a one to three chance that the pancreas is diseased, and in case of pancreatic disease about one in fourteen shows sugar in the urine. The truth is well expressed by Sladden when he says, "viewed arithmetically, glycosuria is not a sign of great diagnostic value." If, however, glycosuria either spontaneous or provocative be present together with other confirmatory evidence of pancreatic disease the symptom then acquires a greater value.

It must be remembered that the liver is also concerned to a very important and intimate extent with carbohydrate metabolism, and tests for alimentary glycosuria have long been employed with a view of estimating the functional integrity of that organ. Under a previous chapter these tests have been given and discussed.

It must be perforce admitted that the question of applying the so-called carbohydrate tests for provoca-

tive glycosuria to the elucidation of pancreatic function is one which for the present must be left open.

It would appear rational to assume that the presence of glycosuria after the provocative tests indicates either hepatic or pancreatic insufficiency or both, and such tests are never in themselves sufficient to elucidate the problems involved, but where they are corroborated by other more specific evidences of insufficiency, they assume an importance in diagnosis of no mean value, an importance which is entirely lacking to them when interpreted alone.

GENERAL CONCLUSIONS CONCERNING THE TESTS FOR PANCREATIC INSUFFICIENCY

It is certainly true that no one functional test of pancreatic activity so far devised constitutes an absolute or pathognomonic sign of disease of this organ. It is quite natural that this should be so in view of the manifold functions of the pancreas.

In other words it is often a very difficult question to determine in a given case whether the pancreas is diseased or insufficient at all, much less to make out by means of the most complete and comprehensive semeiological study the precise nature and extent of the pathological processes.

The whole subject of pancreatic clinical pathology and diagnosis and that of tests for functional activity of the organ is in its infancy.

Nevertheless the value of those functional tests so far devised is considerable and it is the duty of clinicians to apply them in practice to such an extent that their individual and collective worth or lack of worth may be definitely determined.

CHAPTER IV

TESTS OF HEART FUNCTION

GENERAL CONSIDERATIONS

In recent times a considerable transformation has occurred in the viewpoint of clinicians toward the cardiopathies and cardiac pathology. A few years ago all cardiopathology was discussed in terms of anatomical lesion. The chief interest lay in the exact localization and delimitation of the lesion. The tendency at the present is to bring more and more into the foreground the idea of functional capacity of the organ. Recent studies in cardiac physiology and pathology have shown that the fundamental factor is the muscle itself rather than its innervation as was before believed. It has been likewise shown that the function of the cardiac muscle is a complex one and that at least five subdivisions of function may be made (Englemann). These may be enumerated. The heart muscle possesses the power of originating contractile impulses (impulse formation, chronotropic function); it possesses the faculty of susceptibility to the receipt of these functions (excitability, irritability, bathmotropic function); it is endowed by means of the conducting system of fibers including a histological differentiated tissue, the bundle of His with power to transmit these impulses from the point of their formation, the

sino-auricular node to the cardiac muscular fibers (the conducting function, conduction, dromotropic function); it possesses the fundamental power of contraction (contractility, inotropic function), and finally, the muscle possesses that vital function by which it normally refuses to dilate beyond a certain point (tonicity).

A perfect method of estimating cardiac function would be one in which all these five functions of the cardiac muscle could be separately measured. The function of the entire organ would be then their arithmetical sum if all possessed some degree of integrity, or their algebraic sum if certain of them were below a normal point which might be diagrammatically represented by zero. But as a matter of fact we are far from being able to accomplish such an estimate of cardiac function at the present time.

There are many instances, however, in which a careful physical examination of the organ together with the use of special methods of cardiac investigation which have come into use in recent years (sphygmograph, electrocardiograph, sphygmodynamometer, the X-ray) will enable the physician to conclude that one or more of the five functions of the cardiac muscle are deficient.

When some or all of these functions have become so insufficient and incompetent that the occurrence of heart failure (asystole) is imminent, the symptoms of the condition (cyanosis, decompensation, edema, signs of venous congestion) become so patent and evident that the presence of cardiac insufficiency is simple to recognize from a study of the physical signs and the symptoms.

This stage of true insufficiency may be called a terminal stage of the cardiopathies no matter what the

anatomical lesion may be in a given case. This stage is known, however, to be preceded by a long period of latency in which cardiac insufficiency, if it is present, cannot be so easily discovered and has to be looked for in order to be recognized.

It is the desire of the clinician to increase his powers of observation, to so lengthen as it were his cardiac vista, that he may be enabled to recognize the earliest signs of cardiac incompetency. It is for this very evident reason that tests for estimating the integrity of heart functions have been devised. As yet no one of them has succeeded in providing an entirely adequate means of obtaining this much to be desired end, nevertheless several interesting and valuable methods have been developed. The work of the past gives promise of future developments and improvements in this extremely important domain of functional diagnosis.

To Rosenbach is generally given the credit of insisting upon the necessity of devising proper tests by which the functional integrity of this most important organ, the heart, might be measured.

The various methods of testing the cardiac function may be divided into a few classes. The first and largest group includes those tests which depend upon the reaction of the heart muscle to various types of exertion active or passive. There is a second and much smaller group based upon the behavior of the heart to reflex stimulation. A third and extremely insignificant group includes but one test, based upon the supposition that sodium chloride elimination is effected by cardiac insufficiency. A fourth group includes modern clinical and instrumental methods of investigating cardiovascular conditions so far as they are concerned with the question of elucidating heart functional power.

The following synopsis shows how the various tests are to be placed in the four categories above mentioned.

I. Reaction to muscular exertion active or passive as a basis for estimating cardiac function.
   1. The staircase test.
   2. Graupner's test.
   3. Mendelsohn's test.
   4. Katzenstein's test.
   5. Herz's self-checking test.
   6. Gymnastic resistance test.
   7. The Russian test—"Holding the breath" test.
   8. The Venous pressure test.

II. Application of cardiac reflex estimations in determining heart function.
   Merklen's test.

III. Estimation of sodium chloride elimination as a test of cardiac sufficiency.
   Vaquez-Digne test.

IV. Modern clinical and instrumental methods of investigating cardiovascular conditions: their applicability to estimating cardiac function.
   1. The sphygmomanometer as an index of cardiac function. (Work velocity ratio; sphygmobolometry sphygmobolography, energometry, etc.)
   6. Röntgenoscopy and Röntgenography as indices of cardiac function.
   7. Sphygmocardiography and electrocardiography; their relation to cardiac functional capacity.

I. REACTION TO MUSCULAR EXERTION ACTIVE OR PASSIVE AS A BASIS FOR ESTIMATING CARDIAC FUNCTION

The majority of the methods so far suggested for estimating heart functional power have consisted in

the subjection of the patient to a certain measured degree of physical exertion followed by the systematic observation of the phenomena produced by the exertion as compared with conditions carefully ascertained prior to the beginning of the test.

All these methods, however, have the common objection that the same work prescribed to different individuals, will under normal circumstances produce quite different results, according to certain circumstances, among which are the size and general muscular strength of the individual, the usual mode of life with respect to physical exertion, the condition of the nervous system, etc. The result, therefore, of exertion tests may not always be comparable even in healthy persons. If these factors can be properly estimated and provided for the exertion tests are rendered more certain and hence more useful.

Herz has emphasized the fact that the cardiac phenomena produced by exertion tests are varied by the type of effort attempted: whether, for example, the movements are rhythmical and gymnastic, whether they are resisted or not, and especially whether the muscular groups called into play are weaker or stronger. A much higher rise of blood pressure is produced by the effort attempted by a weaker set of muscles than the same operation performed by a stronger set. Grebner and Grunbaum have contended that the increase in blood pressure produced by muscular contractions is inversely proportional to what may be termed the specific energy of the muscles employed in the effort.

The influence of psychic factors in varying the results of muscular tests has always been recognized (Kornfelds). The same may be said of the nerve factors. All these facts tend of course to render uncertain the results obtained by the exertion tests (pulse

rate, blood pressure), but even with these defects this type of method of estimating cardiac function is of practical value.

The chief points taken into consideration in this type of test are the rate of the pulse, the blood pressure (systolic and diastolic) and the area of cardiac dullness or size of the heart (percussion, röntgenography).

As long ago as 1833 Donnell showed that the pulse rate is normally slower in the recumbent than in the semi-erect and erect positions. Christ in 1894 [1] proposed to register the exact pulse rate after exertion with the sphygmograph provided with a time marker. He likewise invented an apparatus by which the patient could undertake a measured amount of exertion on a steppage machine. Rosenbach in the same year emphasized the importance of noting the condition of the skin after the performance of the exertion. It was his belief that the skin remains dry if the heart is competent but becomes quickly moistened with perspiration if there is cardiac insufficiency. He explained the increased sudation on the ground that the excretory function of the skin is called from the list of reserve forces to compensate as far as possible for the cardiac inadequacy. This is probably not the correct interpretation of the phenomenon since sweating itself requires the expenditure of force. It is probably due to vasomotor causes.

1. The Staircase Test. Selig's Test [2]

Technique.—Count the pulse and take the systolic pressure. Have the patient ascend a flight of twenty steps, rapidly. Count the pulse and take the systolic pressure after the ascension.

[1] Archiv f. klin. Med., 1894, LIII, 1902.
[2] Prag. med. Wchnschr., 1905, XXX, 418, 432.

Under normal circumstances, there is an increase in the pulse rate of 20 beats per minute on an average and a rise of blood pressure of 8 millimeters of Hg.

If the myocardium is insufficient there will be an increase in the pulse rate of 30 beats per minute or more. The blood pressure rise will be slower and average about 6 mm. of Hg. The rise may be followed rather suddenly by a fall below normal or the preliminary rise may be absent.

The length of time required for recovery to the normal systolic pressure may be taken as a measure of the amount of cardiac insufficiency present.

The staircase test on account of its simplicity is often employed by clinicians.

The "hopping test" is a modification of the Selig test, which has been used for years as a routine method of eliciting a latent cardiac insufficiency. The patient is instructed to hop 20 paces on one foot and a comparison is then made between the pulse rate before and after the exertion.

One serious objection to the "hopping test" is that the actual amount of work performed by the individual to be tested cannot be computed. In the method of climbing stairs, the amount of energy expended can be approximately known. The amount of work done in foot pounds will be equal to the product of the weight of the individual in pounds into the number of feet ascended.

The advantage of this simple test is that it can be performed without any special apparatus, which is the chief objection from a practical standpoint to some of the functional cardiac tests which have been suggested.

2. *Graupner's Test* [3]

Graupner found at Nauheim in observing the reaction of patients after the exercises carried out as a part of the treatment of cardiopathies, that persons with weakened hearts showed a different type of reaction from those with normal or nearly normal myocardia.

Under normal circumstances, as is well known, the pulse rate and the systolic blood pressure rise after exertion, returning to normal after a fairly short interval. If the exertion is sufficiently prolonged and arduous they may fall below the normal. Graupner discovered that after the pulse rate has risen and again fallen to normal after an exertion, the systolic pressure rises gradually to a maximum, which is reached in a few minutes, usually about six, declining to normal in about 18 to 20 minutes. The rise of blood pressure following the pulse rise is called the normal *erholung*. In weakened hearts, even if the weakness is slight, Graupner found that the *erholung* occurs but it is less in amount than normal and is delayed beyond the normal interval, usually to about 12 minutes. If the heart is seriously weakened the *erholung* may be absent altogether, the blood pressure declining from the start then gradually rising to normal. In normal cases the pulse reaches its normal in 5 to 10 minutes.

Technique of Graupner's Test.—A Zuntz Ergometer [4] of the bicycle or weight and pulley type is used in conducting the test. The patient turns a wheel which is supplied with a brake and adjustments for measuring the amount of work expended. Tests are

[3] Berl. klin. Wchnschr., 1902, 174; Deutsch. med. Wchnschr., 1906, XXXII, 1029.

[4] Centrbl. f. Physiol., 1898, 502.

made on successive days at the same hour. The work is therefore done by the same muscle groups. It is important not to carry the work to the point of exhaustion or strain. Mental excitement must be absent. The pulse rate, blood pressure and size of the heart are noted before and after the test.

Arm muscle work may be substituted for thigh muscle work on the same machine and this was done by Graupner in his later researches.

Cabot [5] and Bruce have recommended using a measured amount of stair climbing, which of course is a more practicable and generally useful method. They estimate the amount of work in foot pounds which is readily computed by multiplying the number of pounds the individual weighs by the number of feet ascended.

Graupner came to the following conclusions as a result of his rather extensive investigations: If the blood pressure remains constant after the exercise the heart muscle is sufficient. If the blood pressure falls after the exercise there is cardiac insufficiency. If the blood pressure rises but returns to normal there is compensatory sufficiency. If the blood pressure rises then rapidly falls without a tendency to subsequent rise the heart muscle is fatigued.

Graupner stated as his belief that if the pulse is accelerated and the patient becomes short of breath after the performance of work equivalent to 45 to 300 kilograms the heart is manifestly insufficient.

Several authors have corroborated Graupner's view that a persistent tendency toward a fall of blood pressure after the exertion denotes cardiac insufficiency. According to Graupner's later observation persons with normal hearts can perform arm muscle work on the ergometer equivalent to from 3,000 to 20,000 kilo-

[5] Amer. Jour. Med. Sc., CXXXIV, 1907, 491.

grams per hour. If the figures fall below 1000 kilograms per hour there is cardiac insufficiency. If measurements are made every half minute after exercise it was found that the amount of variability in the blood pressure corresponds with the insufficiency of the heart, in other words, that the greater the heart weakness the greater are the variations in the pressure and the longer the time required for the *status quo* to be restored. This does not wholly occur for 30 to 35 minutes.

Some observers have found that a lowering of the blood pressure after exertion may be found in trained athletes and according to the terms of the test this should denote cardiac insufficiency. But as Hirschfelder [6] states, "the heart of the trained athlete is habitually throwing out an amount of blood suited not to the needs of the moment but to the needs of the periods of exercise to which he has accustomed himself." The systolic output is above normal when the exercise (and hence the increased production of CO_2) is slight. The heart is then able to take care of the excess of CO_2 production in exercise without increasing the output and hence the vasodilatation in the muscles is the only factor influencing the blood pressure. When the exercise becomes severe the other mechanisms begin to play a rôle.

Also in certain patients with diseased hearts the blood pressure has been found to rise, it is claimed, because of high pressure stasis. This rise, however, comes later than in normal cases.

Cabot and Bruce as a result of their trial of Graupner's test in seventy-five experiments believe that it is reliable. They say "the main outlines of Graupner's contention can be easily verified by anyone. Run

[6] Hirschfelder, Diseases of the Heart and Aorta, p. 286.

quickly up two flights of stairs and then stop and count your pulse. After the immediate acceleration is passed or during the slowing of the pulse following it you will note that the heart beat and the strength of the pulse become markedly exaggerated. One feels the thump thump of the heart against the ribs much more strongly after the pulse has almost or quite reached its normal rate than during the period when the pulse is most accelerated. . . . As regards the phenomenon designated by Graupner as the normal *erholung* we can verify his findings and we likewise agree with him in the results of our experiments upon seriously weakened hearts. In some of the cases believed by us from ordinary examination of the heart to be normal there was considerable variation from the ordinary curve of blood-pressure after exertion. Cases of valvular disease with good compensation showed, as might have been expected, a normal curve."

Tests like that of Graupner in which blood pressure estimations are used as a criterion of cardiac function are founded upon observations of Masing[7] and others that the normal blood pressure rises during exercise and falls immediately afterward.

When a normal individual exercises with chest weights, for example, the blood pressure may rise 10 to 30 mm. of Hg. If the individual is arteriosclerotic the rise of blood pressure may go to 40 to 60 mm. of Hg. and outlast the exercise a variable length of time.

Bauer in employing this test used a stationary bicycle and this is a good method because the blood pressure estimations can be easily taken in the arm during the performance of the exercise. According to Bauer the bicycle test gives for normal individuals a rise of 5 to 10 mm. of Hg., while in those with cardiac insufficiency

[7] Deutsch. Arch. f. klin. Med., Leipz., 1901, LXXI, 253.

there may be a fall of equal degree (5 to 10 mm. of Hg.).

The great difficulty with this test is that it has been found that in trained athletes the blood pressure may fall instead of rising at the commencement of mild exercise and the fall may last for a considerable period, thus making the reaction of the strong man somewhat similar to that of the weak. The proper interpretation of this fact has been given.

As Hirschfelder states all functional tests of cardiac efficiency if based upon mathematical changes in pulse and blood pressure may lead to ambiguous results. This is no objection, however, to the application of the tests but only to their too strict interpretation. The appearance of the patient after the performance of the physical tests is of course extremely important. Accelerated or labored breathing, holding the breath, dilatation of the nostrils, drawing in of the corners of the mouth, darkness or pallor of the cheeks, sweating, palpitation and so forth, all these are signs of cardiac insufficiency more important perhaps than the mathematical results of individual tests.

According to Hirschfelder the most reliable numerical criterion of cardiac efficiency is whether a given strain causes the heart to diminish in size (increase in tonicity) or to dilate (decrease in tonicity—overstrain).[8]

3. Mendelsohn's Test [9]

Technic.—The pulse is carefully counted in the standing, sitting and recumbent postures and the figures noted. This may be repeated several times and

[8] Hirschfelder, loc. cit., 199.

[9] XIX Kongr. f. Inn. Med., 1901.

an average taken. The person to be tested then performs muscular work upon a Gaertner ergostat by means of which the amount of work may be measured.[10] The Gaertner ergostat is an instrument not easily secured and for this reason the original test is not much employed. By the simple method, however, of Cabot and Bruce above mentioned of having the patient perform a given amount of work in stair climbing which can be easily calculated, the reaction of the pulse rate and the return of the latter to normal, which is the basis of the Mendelsohn tests, can be readily estimated. After the performance of varying amounts of work the patient assumes the recumbent posture immediately and the time is noted which is required for the pulse to return to the normal figure previously ascertained for that posture.

The first criterion of the Mendelsohn test is based on the principle that when the heart is healthy or well compensated, a transition from vertical to horizontal position is accompanied by a slowing of the pulse of 10 or 12 beats per minute. If the heart is insufficient or decompensated an opposite condition may prevail, namely, the pulse becomes quicker in the recumbent posture or tends to remain constant.

Mendelsohn contended that if there is not a well marked difference in the pulse rate between the erect and recumbent postures the heart is incompetent.

The second criterion suggested by Mendelsohn depends upon the principle that the competent heart is able to return immediately to a normal when resting after a strain. He suggested, therefore, that an estimate of the functional capacity of the heart can be obtained by noting the degree of facility displayed by the organ to return to normal conditions after meas-

[10] Allg. Wien. Med. Zeit., 1887, Nos. 49 and 50.

ured exertion in which extra cardiac energy is called into play. Mendelsohn found that a normal heart after performing work equivalent to 100-200 kilograms returns immediately to the normal with rest in recumbent posture. After 500 kilograms of work the normal heart is accelerated somewhat for a varying period of time. If, however, there is cardiac insufficiency very much smaller amounts of muscular exertion prove excessive and disturb the pulse rate. A disturbance of rate with failure to return immediately to normal following the expenditure of 25-50 kilograms of work denotes cardiac insufficiency.

A Variant of Mendelsohn's Test.—When a normal individual rises from the reclining to the standing position the heart rate is accelerated, but it is usually stated that the increase ought never to be more than 20. Beyond 20 one has the right to assume that the myocardium is insufficient.

This test from its extreme simplicity has been much used and is capable of giving some valuable information. But nevertheless, under some circumstances it fails to do so; for example, under conditions where the psychic rôle may play a part. Here the increase of pulse rate in a normal individual, that is normal so far as the myocardium is concerned, may be inordinate and out of proportion. Hirschfelder [11] says that persons with enteroptosis may give a false increase.

4. The Katzenstein Method [12]

Katzenstein found from animal experiments that ligature of peripheral arteries produces an increase in

[11] International Clinics, Vol. IV, 1910, p. 39.

[12] Deutsch. med. Wchnschr., 1904, No. 30, p. 807; also, ibid., 1907, XLIV, No. 16.

the general blood pressure without change in the pulse rate. In animals with weakened hearts he demonstrated that ligature of peripheral arteries produced other results, namely increased pulse frequency and irregularities in the blood pressure. He therefore proposed applying the principle involved to clinical medicine as an aid to determining the functional capacity of the heart muscle.

His method consists essentially in making compression upon peripheral arteries (the femorals) so as to shut off the circulation in the lower limbs, and observing the effects upon the pulse and blood pressure. The author of the test found in cases of cardiac insufficiency a lowering of the blood pressure and a simultaneous increase in the pulse rate, both of which deviations from the normal appeared to maintain a proportionate relation to the incompetency of the heart muscle.

It has been proposed to substitute an Esmarch bandage for digital compression of the arteries, thereby doing away with the necessity of an assistant.

Technic of Katzenstein Test.—Sometimes called also the Marey-Katzenstein-Shapiro Test.

The patient is put in a reclining posture and the pulse rate and blood pressure taken. Pressure is then made for two and one-half to five minutes over both femoral arteries in the groins by means of the fingers of an assistant or by elastic Esmarch bandage, or according to Morelli by inflatable rubber stockings. The pulse rate and blood pressure are again recorded.

In normal individuals with sufficiency of the myocardium the pulse diminishes in number. The blood pressure rises 5 to 15 mm. of Hg. With sufficient but hypertrophic hearts the pulse diminishes or remains the same. An increase of 15 to 40 mm. of Hg. takes place in the blood pressure.

In cases of moderate latent cardiac insufficiency the blood pressure remains unchanged. The pulse rate is unchanged or increased. In higher grades of cardiac insufficiency the blood pressure sinks and the pulse rate increases.

For practical purposes it may be said that with sufficiency of the heart muscle the pulse remains unchanged or diminishes in number and the blood pressure rises. If the pulse increases and the blood pressure remains the same or falls after the Katzenstein test the heart is insufficient.

Norris [13] made an investigation in 1907 with a view of determining the adaptability of Katzenstein's test to clinical use. He found that generally speaking the results were accurate and confirmatory of its author's findings but many exceptions were noted. Some of the cases, many in fact, of cardiac weakness which responded positively to the test did so in an extremely equivocal manner, leaving practically the final determination a matter of personal equation on the part of the investigator.

As a corroborative test the method of Katzenstein appears to possess some value, but as an independent test of cardiac sufficiency or insufficiency no great dependence can be placed upon it. The method should be used with caution in cases of severe cardiac weakness where it may prove to be actually dangerous.

5. *Herz's Self-Checking Test.*[14] *Selbst-Hemmungs Probe*

Technic.—The patient is placed in a sitting posture and remains so until the pulse rate has become con-

[13] Blood Pressure and Clinical Applications, Phila., 1914, p. 145; International Clinics, 1907, I, 17s, p. 66.

[14] Deutsch. med. Wchnschr., 1905, 31, XXXI, 215.

stant. He is then directed to contract all the muscles of hand and forearm with all his force and to flex and extend the forearm with all possible force, performing the motions slowly, paying strict attention to the performance and endeavoring to antagonize his movements as forcefully as possible.

In healthy persons the pulse rate is unaffected by this maneuver, whereas in persons with a weak heart the rate increases 5 to 20 beats per minute.

This test has been found to possess a certain degree of reliability but it does not possess so absolute a value as was originally ascribed to it by its author. Sometimes healthy persons give a positive reaction. Hirschfelder believes that the vagus plays a part in it and that the results are not altogether indicative of cardiac output and vigor.

6. *Gymnastic Resistance Test. Herz-Haranchipy Test*

This consists in having the patient execute three types of resisted movements. First a movement of flexion-extension of the forearm, the patient being seated. Second, a movement of separation-approximation of the thighs in sitting posture. Third, a movement of abduction-adduction of the extended lower limbs, the patient seated. All of these motions are resisted equally. A slight rest is given between each series. The whole test lasts 25 to 30 seconds. Prior to the movements the systolic blood pressure is taken. While the movements are being executed and during repose the blood pressure is retaken. In a normal individual there should be a variation in the blood pres-

sure as a result of the exercises of not more than 10 to 15 mm. of Hg. In cases of cardiac insufficiency it reaches 20 to 30 mm. of Hg.

7. *The Russian Test.*[15] *"Holding the Breath" Test*

This simple test for estimating the integrity of the cardiac muscle has been in long use empirically. We have called it the Russian test because Herz has mentioned the fact that he could not find any specific mention of it in the literature but knew that it was commonly employed by certain Russian physicians.

The test is well called "Holding the Breath" test since it consists simply in directing the patient to stop breathing for as long a time as possible. This maneuver puts a considerably added strain upon the myocardium, particularly the right ventricle. Great variations of the length of time in which the breath can be held by different persons are found, but any marked limitation of the time during which a person can inhibit the act of respiration indicates cardiac insufficiency. If the period of voluntary apnœa is less than 15 seconds the myocardium may be considered insufficient.

8. *The Venous Pressure Test. Schott's*[16] *Test*

The principle of the Schott test depends upon the fact that in health if the arm is elevated to an angle of 60 degrees with the patient in a recumbent posture and making no other exertion the venous pressure increases only .5 cm. of H_2O or sometimes may remain

[15] Die Herz Krankheiten, Wien, 1912, p. 125.
[16] Deutsch. Arch. f. klin. Med., 1912, CVIII, 537.

stationary or even fall. If, however, the cardiac muscle is insufficient, a rise in the venous pressure takes place which may even be considerable (4 to 7 cm.). According to Schott, any reading above 3 cm. denotes cardiac insufficiency.

Several methods have been devised to determine venous blood pressure. Von Frey and Gaertner considered that the venous pressure can be determined by considering it equal to the height above the angle of Ludwig at which the veins of the hand are seen to collapse when the arm is raised. Von Recklinghausen used an apparatus whereby the vein could be compressed by inflating a small rubber capsule provided with a glass window in the top of a rubber dam floor with an opening in its center. The dam is coated with glycerine to insure perfect apposition to the skin. It is placed over a vein on the back of the hand or wrist and the system inflated until the vein is seen to disappear, at which point the pressure is read off on a water manometer. Eyster and Hooker modified the method by using an aluminum chamber with a glass top, the two ends concave to avoid pressure on the vein. The normal venous pressure obtained by this instrument at the sterno-xyphoid articulation is 5 to 10 cm. of H_2O. In cardiac cases it may rise to 27 cm. or more. The pressure in the lip capillaries may be estimated by using the point of blanching as the criterion. The study of venous pressure is of some importance as an index of accumulation of blood in the veins and may therefore become to some extent an index of heart failure.

II. APPLICATION OF CARDIAC REFLEX ESTIMATIONS IN DETERMINING HEART FUNCTION

Merklen's Test

The best known cardiac reflexes are those of Abrams and Livierato.

Abrams' reflex consists of a diminution of the area of precordial dullness following energetic friction over the heart. Livierato's reflex consists of an increase of the area of cardiac dullness following percussion over the epigastric region. In Abrams' reflex the left ventricle is chiefly affected and in Livierato's reflex the right ventricle. The heart is so much less meiopragic (weakened) in proportion to its capacity to give a positive Abrams and a negative Livierato.

Technique of Reflex Test.—Map out the area of precordial dullness carefully by light percussion and mark with dermographic pencil. Make precordial friction for one minute, using rough cloth or a rubber eraser. Follow this by rapid percussion of the precordial area. After three to five minutes' wait, map out the area of dullness by light percussion. If the reflex is normal, the area will be smaller than before.

In using Livierato's reflex the technique is the same to determine the area of cardiac dullness. The reflex is elicited by making a series of rapid rather forceful strokes for one minute over the median line of the abdomen. After three minutes' interim, the area of dullness is again made out and if the reflex is positive the right border of cardiac dullness will be found increased. The two reflexes should not be applied to the same patient on the same day.

III. ESTIMATION OF SODIUM CHLORIDE ELIMINATION AS A TEST OF CARDIAC SUFFICIENCY

Vaquez-Digne Test

This test is based upon the supposition that the elimination of sodium chloride is affected by the sufficiency or insufficiency of the heart muscle. In individuals in whom the integrity of the heart muscle is normal any excess of salt ingested should be promptly eliminated from the circulation had passed out through the kidney. In cases of cardiac insufficiency even when latent, it is contended that the salt elimination is defective.

In applying this test the individual is put for some days on a fixed sodium chloride ration and when an equilibrium is established the amount of salt injected is doubled and a quantitative estimation of sodium chloride in the urine made. In cases of cardiac insufficiency there will be defective elimination and if the diminution of function is considerable there may be œdema and signs of partial decompensation set up. The integrity of the kidney function must be previously determined.

IV. MODERN CLINICAL AND INSTRUMENTAL METHODS OF INVESTIGATING CARDIOVASCULAR CONDITIONS: THEIR APPLICABILITY TO ESTIMATING CARDIAC FUNCTION

1. Sphygmomanometer as an Index of Cardiac Function. Work-Velocity Ratio

The sphygmomanometer is the instrument in vogue of our day. The chief value of this instrument is to

register the height of the blood pressure and since its introduction it has no doubt contributed to a clearer differentiation of states of hyper- and hypotension. Like the sphygmograph, it is teaching physicians to become more expert in the use of their sense of touch and just as the latter instrument (sphygmograph) taught physicians to differentiate the arhythmias without the use of the instrument in many cases, so, too, it may come to pass that careful comparison of palpatory pulse estimations of pressure with instrumental readings of pressure carried out day by day in cases of hyper- and hypotension will finally educate the physician to make correct deductions in many cases without the use of the instrument. There will always remain a certain proportion of cases, however, in which, owing to various physical factors, an accurate digital estimation of the systolic blood pressure is impossible. A distinguished clinician who has cultivated this perception to a remarkable degree is quoted as saying: "I can estimate the blood pressure with the fingers alone quite accurately in about eight cases out of ten, but those in which it is of real importance are always the other two."

Modern sphygmomanometry will, however, do something more than show the variations in the systolic blood pressure. Recently, since the introduction of the auscultatory or auditory method of using the sphygmomanometer, the method of Korotkof, a more accurate means of finding the exact diastolic pressure has been found than could be had by means of the older visual method with the vertical mercury or other manometers.

With accurate data concerning the systolic and diastolic blood pressure, we are in a better position to interpret results in terms of cardiovascular function

than we are by means of the systolic pressure alone.

In order to fully appreciate just what may be expected from such data as the above in the interpretation of cardiac function, we must bear in mind of course a few simple physiological facts.

The blood pressure, that is, the systolic blood pressure, depends mainly upon the contractile powers of the heart muscle which enables it to pump the blood into the arteries, against the peripheral resistance caused by the friction of the blood on the vessel walls. The peripheral resistance depends upon the tonicity and physical state of the vessel walls. Under normal circumstances the elasticity of the coats of the arteries provides for a continuous instead of an intermittent flow of blood which would be the case if the arteries were rigid tubes.

The systolic or maximum pressure will approximately show the actual pressure or work developed by the heart at the moment of systole. The diastolic or minimum pressure will show the degree of the peripheral resistance which the heart has been able to overcome and which is maintained in the peripheral circulation during the time of heart refilling. The difference between the highest and the lowest pressures in the larger arteries, that is the difference between systolic and diastolic pressures, is known as the pulse pressure. The pulse pressure, therefore, is the measure of the amount of force exerted by the heart in maintaining the blood pressure over and above the arterial or peripheral resistance. To this extent then the pulse pressure is a measure of the pumping capacity of the heart and hence is of some importance in estimating the state of cardiac function.

Gibson has called attention to the fact that there are certain normal arithmetical relations which are

discoverable in a study of the three factors—systolic pressure, diastolic pressure and pulse pressure. The relation of the diastolic pressure to the systolic pressure is normally as two is to three. The relation of the pulse pressure to the systolic pressure is as one is to three. If the systolic pressure is 150 the normal diastolic pressure will be approximately 100. With a systolic pressure of 150, the normal pulse pressure will be 50. Of course these figures represent approximate and not absolute relations. They are of some service in estimating cardiac function because pathological relations become evident and the presence and also to a certain extent, the degree, of cardiac overload may be appreciated.

The normal arteries will apparently withstand a continual variation in pressure, that is a pulse pressure of 35 to 50 mm. of mercury without deterioration. Any great increase of the pulse pressure over these figures is pathological and at least indicates cardiac overload and consequently justifies suspicion that perhaps the myocardium may not long succeed in maintaining it. The heart manages to do so by undergoing hypertrophy and when this has been accomplished the organ may be regarded as at least a *locus minoris resistentiæ.*

A quantitative idea of the undue stress may be obtained by taking the difference between the normal and pathological pulse pressure. When this is multiplied by the pulse rate and this by 60 (hour) and again by 24 (day) a concrete idea may be gained of the enormous excess of energy required to be expended by the heart in a day to overcome pathological peripheral resistance.

Such a case, however, may go on and on and we have no exact method of predicting just when the break will come, with its attendant consequences of cardiac in-

sufficiency. As it approaches, however, the diastolic pressure will fall, indicating the approaching collapse of the cardiovascular mechanism. The larger the pulse pressure in relation to the diastolic pressure, the greater the strain on the heart will be and the more imminent, therefore, is decompensation. A high systolic pressure with a relatively low diastolic pressure indicates, therefore, impending collapse of the heart muscle. If after treatment the diastolic pressure rises and the pulse pressure falls the indication is that recompensation is taking place.

Technique of Sphygmomanometry.—A brief account only of the method of obtaining the systolic and diastolic blood pressure will be given. A more complete description is properly found in texts devoted to the subject of blood pressure. The arm band is applied to the bared arm above the elbow by placing the broad end containing the rubber bag over the region of the brachial artery. Wrap the band bandagewise around the arm and tuck in the narrow end. Connect up the indicator and pump. Apply the sphygmometroscope or ausculoscope or stethoscope over the brachial artery at the bend of the elbow. Increase the pressure until all sounds are gone, then gradually admit air. The sounds which are heard are divided into four phases. First, a loud clear-cut snapping tone. This is caused by the first and the early pulse waves that break through the constriction and its beginning represents the systolic pressure. Owing to the greater sensitiveness of the ear than the fingertip it is usually heard some 5 to 10 mm. above the point where the radial pulse is first felt. In normal cases, it is said to last during the fall of about 14 mm. of pressure. Second, a murmur or succession of murmurs lasting during the fall of about 20 mm. This phase is not

always present and if absent the first and third phases merge into one another. Third, a clear tone resembling the first, sometimes less well marked but often louder. This lasts during a fall of about 5 mm. Fourth, a rather sharp transition from the loud to the dull tone recently proved to be the time of diastolic pressure.

As there had been considerable discussion as to whether the diastolic pressure corresponded to the beginning or ending of the fourth phase, Warfield undertook its investigation some years ago through a series of animal investigations and comparison of cases with accurate graphic records. These have been confirmed by others and the point now seems to be settled, that diastolic pressure coincides with the beginning of the fourth phase.

2. *Functional Tests Based on Direct Blood Pressure Determinations*

The Cardiac Efficiency Factor of Tiegerstedt.— The pulse pressure shows approximately the systolic output in energy and the velocity of the blood stream will be the product of this energy into the number of cardiac cycles (pulse beats) per minute. In other words, pulse pressure (PP) times pulse rate (PR) will equal velocity of flow.

Since the interventricular pressure is almost constant throughout systole, it is evident that the work done by the heart is tolerably constant throughout the period. The work done by the heart in a unit of time will be the product of its maximum energy, systolic pressure (SP), multiplied by the pulse rate (PR), multiplied by the duration of systole. Since the interventricular pressure is constant, the factor duration of systole may be eliminated and work done equals product

of SP $\times$ PR (systolic pressure times pulse rate). The reason why interventricular pressure is constant is because of the fact that the heart liberates all available energy at each contraction. A concrete example will readily show how the velocity work ratio is obtained. If the SP = 130, DP = 85, then PP = 45. If PR = 70 then PP 45 $\times$ PR 70 = 3,100 (velocity), and SP 130 $\times$ PR 70 = 9,100 (work), the ratio then becomes

$$\frac{\text{PP}}{\text{SP}} = \text{Blood pressure coefficient (Tiegerstedt).}$$

$$\frac{\text{PP} \times \text{PR}}{\text{SP} \times \text{PR}} = \frac{\text{Velocity}}{\text{Work}}.$$

This is the velocity work ratio or coefficient of heart pumping efficiency. The velocity work relation in this example is one to three and this is about the normal ratio. Expressed in percentages, it varies normally from 25 to 35%. Increase in this ratio may indicate cardiac insufficiency.

The Cardiac Strength, Cardiac Weakness Ratio of Goodman and Howell.—These authors have studied the duration of the four-tone phases of auscultatory blood pressure estimation in a series of normal and pathological cases. Their test is based on the ratio of these phases to the pulse pressure and to one another. They set forth their views as follows:

1. "The first phase or tone phase serves principally as an index as to how far the pressure must fall before the blood current can be sustained past the obstruction in the vessel caused by the cuff at a sufficient velocity and for a sufficient duration to produce the murmur. Hence the information it affords is of negative rather than of positive value. In other words, its normal

duration is of no value but an increase or decrease in length is of importance.

2. The second or the murmur phase seems to be especially dependent upon cardiac effectiveness, for it is in this phase alone that the individual sounds possess a distinct element of duration and this protracted energy, for so it must be regarded, must evidently come from the heart.

3. The third phase or second tone phase depends not alone on cardiac efficiency but also on the character of the vessel wall. The more sclerotic the vessel and the greater the cardiac hypertrophy, the more favorable are the conditions for the production of a clear tone.

4. As the fourth phase or dull tone may be produced by a resilient vessel, receiving a normal pulse shock, or by a rigid vessel receiving a weakened shock, its interpretation is more difficult. If our assumptions are correct it is evident that increases in the second and third phases are dependent on cardiac strength and circulatory deficiency, while the first and fourth phases suffer increase when there is cardiac weakness. Furthermore, in dealing with increases or decreases in any particular phase it is important to know at the expense of what adjacent phase this has occurred. It is apparent that an increase in the third phase for example at the expense of the second has not the same significance as an increase of this phase at the expense of the fourth. In the first instance the unit of cardiac strength which we obtain by adding the lengths of the second and third phases has not been materially changed while in the latter it has been increased. For this reason we recommend that the sum of the second and third phases be compared with the sum of the first and fourth phases in order to determine whether the elements of force or those of weakness are predominating.

Aside from the value of the persistence of the fourth phase in aortic insufficiency little of diagnostic value has developed in regard to the length of any individual phase. Advantage has been derived, however, from studying the changes in the sequence reading, specially in decompensating cardiac lesions as the patient improves or not. In these cases changes in the percentages of the various phases are not the only significant feature but internal peculiarities appear. Or to put it another way, sequence readings have a functional rather than an organic significance. Our results uniformly show that with decompensation or circulatory disturbances of lesser degree, the element of heart weakness (the sum of the first and fourth phases) progressively encroaches upon that of heart strength (the sum of the second and third phases). The second phase appears to be the one which is with most difficulty sustained. The fourth phase as weakness gains the ascendency, is usually the first to lengthen the element of cardiac weakness by its encroachment on the third phase, but encroachment of the first phase on the second soon adds its share to the total." [17]

The average duration in mm., the fall of and percentages of the pulse pressure of the different phases in normal individuals are:

	mm.	per cent.
First phase	14	31.1
Second phase	20	44.4
Third phase	5	11.1
Fourth phase	6	13.3

The cardiac strength (second and third phases): cardiac weakness (first and fourth phases) :: 55.5: 44.4.

[17] Amer. Jour. Med. Sci., 1911, CXLII, 336.

Marked increase of the cardiac weakness factors indicates cardiac inefficiency.

Previously to Goodman and Howell's work many observers [18] have recognized that the duration of the second phase of the auscultatory tones indicated cardiac strength. A considerable reduction of its normal percentage of the pulse pressure is therefore taken as a test of cardiac insufficiency.

The Cardiac Overload Factor of Stone.—A paper on the clinical significance of high and low pulse pressure with special reference to cardiac load and overload with a report of 170 cases was presented by Stone at the meeting of the American Med. Assn. in 1912.[19]

The ratio of the pulse pressure to the diastolic pressure representing the load of the heart has since been used as a test for cardiac efficiency. Stone says, "the pulse pressure measures the energy of the heart in systole in excess of the diastolic pressure. For clinical purposes it represents the load of the heart. The myocardiac load may therefore be expressed by the fraction $\frac{\text{pulse pressure}}{\text{diastolic pressure}}$ and under normal conditions is approximately 50%." Anything in excess of this is an overload. The average load in these cases was 71, an overload of 21%. Naturally in this group of cases some did well and some badly. To quote again, "judging from this small series of cases it would appear that when the overload factor exceeds 50% the patient may be in danger of myocardial exhaustion at any time of slight overstrain. As a rule the greater the overload the greater the danger." That is to say, an overload

[18] Forman, Ztschr. f. diet. und physik. Therap., XIII, 809; Fisher: Deutsch. med. Wchnschr., 1908, XXXIV, 1141.

[19] Jour. Amer. Assn., 1913, LXI, 1256.

of 50% or more (the pulse pressure equal to or greater than the diastolic pressure) indicates cardiac insufficiency of a considerable degree with impending decompensation. Whereas an overload of 25% would seem to indicate a mild degree of insufficiency.

Swan [20] has recently published a study of the above four tests with a series of observations on 40 pathological cases. His conclusions are as follows: "It appears to me legitimate from the study of the cases reported to conclude that all four of these factors have some value in determining the efficiency of the myocardium. I am inclined to think at present that the cardiac efficiency factor of Tiegerstedt and the percentage of the pulse pressure formed by the second phase are the most important. A cardiac efficiency factor of 40% or over would seem to point out distinct myocardial inefficiency. A second phase of 30% or under would seem to indicate the same condition. The CS to CW (cardiac strength to cardiac weakness) ratio is less important I think because it so often cannot be determined and again because a small second phase is very frequently made up by a large third phase. On the other hand, CS : CW ratio in which the CW factor is greater than the CS factor is indicative of disturbance of the myocardium, functional if not organic. I am inclined to think at present that the overload factor of Stone is indicative more of peripheral resistance than of myocardial weakness. A cardiac load below 50% as determined by this method giving a negative overload may have some significance, but it will require further study to determine its nature."

[20] Archives Int. Med., 1915, XV, 269.

3. *Sphygmobolometry*

Sahli has claimed for many years that the attempt to estimate the condition of the circulation from sphygmomanometric measurements alone is futile, as the information derived therefrom is static rather than dynamic in character. He therefore believes it would be of great service to endeavor to measure not the blood-pressures, but the energy of the pulse-wave and so, indirectly, the strength of the systole. For this purpose he has constructed an instrument, the sphygmobolometer, which he describes as follows in his exhaustive work on Diagnostic Methods.[21]

As in the measurement of the blood-pressure, the Riva-Rocci cuff with the author's quicksilver manometer is employed. It is necessary that as much as possible of the energy of the pulse-wave shall be transformed into measurable pulsatory movements of the mercury in the manometer. To lessen the friction, a larger tube (5 mm. bore) is used than in the measurement of the blood-pressure. This also enables a float to be placed in the longer limb of the manometer. In order to get a vigorous movement of the mercury a somewhat larger cuff should be used (8 cm. broad). It is convenient to have the cuff connected with a small escape-tube fitted with a stop-cock, so that the pressure in the cuff may be diminished at will, and also to have a stop-cock interposed between the manometer and the bulb. The author employs a four-armed glass tube arranged to connect with the cuff, the manometer, the bulb, and a capillary escape. The cuff is applied to the patient's arm in the usual way, and the pressure grad-

[21] Sahli, H. A treatise on diagnostic methods, edited by N. B. Potter, Philadelphia, 1911.

ually increased; at each increase of pressure, communication between the bulb and the cuff is temporarily shut off, either by using a stop-cock, as above, or by pinching the tube. When a certain pressure is obtained, the mercury in the manometer will be seen to oscillate up and down. Sometimes these oscillations are visible when communication with the bulb is open, but generally they are plainer when the bulb is shut off. Some investigators have employed these oscillations to determine the pressure, e.g., von Recklinghausen and Mosso. They obviously arise from the energy of the pulse-wave, which, at a certain medium point of compression of the brachial artery, is carried through the cuff to the manometer in sufficient strength to set the quicksilver in vibration. If the bulb is connected, the amount of air it contains, of course, materially reduces or even hides the periodic increase of pressure in the air between the pulse and the manometer. The smaller the caliber of the connecting tube and the more rigid its walls, the stronger, of course, are the pulsations. The point of pressure at which the maximum vibrations are set up in the manometer is found not to correspond to that of the maximum nor to that of the minimum arterial blood-pressure, but to lie somewhere between these, not, however, at their exact mean. The author's proposed use of mercury vibrations has nothing in common with conclusions as to the state of the blood-pressure; nor is it related to the attempts of Riva-Rocci and others to employ these vibrations in the determination of the lateral arterial pressure.

Sphygmobolometry is entirely different; it aims to determine, not the pressure, but the work performed by the pulse. The vital energy of the pulse-wave, which is transferred to the cuff, is what sets the column of

mercury in vibration. If that point of compression be selected where these vibrations are at a maximum, i.e., where the transformation of energy is most nearly complete, the work done by the pulse-wave can be calculated after reading off the maximum height of the mercury column. Obviously, however, such conclusions hold good only for one type of apparatus, the results being influenced by the breadth of the cuff, the volume of air-space enclosed, the quality of the rubber, etc. In his original communication the author attempted to announce absolute results concerning the work done by the pulse-wave, but it must be understood that the values thus obtained were always to be multiplied by the constant of the particular instrument. If, however, instruments of the same construction are always employed, as is desirable for clinical work, this constant may be ignored, and at least relatively correct and comparable results be obtained.

One other important point must be noted. Since the maximum excursions of the mercury do not occur at the moment when the manometer shows the maximum pressure, it is obvious that a part of the pulse-wave escapes under the cuff. The author has shown that this fraction is variable, depending upon the shape of the pulse-wave. In order, then, to secure constant conditions the wave must be prevented from escaping in part under the cuff, and as far as possible be forced to exert its energy entirely in the direction of the manometer. This is accomplished by first winding an Esmarch bandage around the arm, either at the elbow or between it and the cuff, tightly enough to obliterate the radial pulse. This device to a certain extent converts that portion of the brachial artery which extends to the lower edge of the cuff into a cul de sac from the aorta (a sort of sphygmoscope).

If, now, the energy of the pulsation be noted at this arterial stump and be measured by the excursions of the mercury, we approximately estimate the energy of the aortic pulse. The instantaneous compression of the venous trunks, caused by the pressure of the cuff, subjects not only the small veins and the arteries, but also the capillaries, to the arterial pressure, and they all pulsate with it. This naturally facilitates the completeness of the transference of energy to the mercury, since not only the small volume of the arteries, but the whole arm stump takes part in the variations of volume. The mechanical conditions of the test are the same in every case; hence it seems proper to assume that practically constant and comparable estimates are obtained, provided, of course, that the pressure of the cuff be adjusted to secure maximum excursions. In making use of this test clinically we must evidently avoid any factor which could artificially alter the energy of the pulse-wave, e.g., pain from an overtight Esmarch bandage, such as to affect the heart action. Caution, experience, and quickness in performing the experiment will, however, usually eliminate this particular factor.

It remains, therefore, merely to measure the extent of the excursions of the mercury, and to make the calculations from the data obtained. For the measurement of the excursions of the mercury, it is sufficient for purely practical clinical or demonstration purposes merely to note with the eye the highest point on the manometric scale reached by the mercury. The distance must, however, be read to fractions of a millimeter. For this purpose a scale giving fifths of a millimeter may be fastened alongside the mercury tube, and by means of a suitable telescope the extent of the excursions be read off from a distance. For more

exact purposes a graphic method of recording the results is preferable. The author uses a float riding directly on the mercury and carrying a writing point, which records the excursions vertically, and without magnification. It writes on a piece of smoked paper, 12 cm. high, fixed in a vertical frame attached to the stand of the manometer near its base. By turning a crank the paper and its frame can be moved horizontally about 6 cm. The manometer is so heavy at the base that this can be done without shaking the instrument. As the writing apparatus, for the sake of stability, must be placed as near the base of the instrument as possible, it becomes necessary to devise some means for transferring the movements of the float from the top of the manometer tube to the bottom. For this purpose a thread is attached to the arm of the float. From it hangs a thin metal wire, bent at its end into a horizontal writing point. This wire is guided by being passed through a glass tube, which is fastened to the instrument. The proper amount of friction can be attained by adjusting this glass tube and a greater or less torsion of the thread.

The technic of the procedure is as follows: the cuff is attached to the upper arm and connected with the apparatus by the 4-armed glass tube. The brachial artery distal to the cuff is compressed by an Esmarch bandage until the radial pulse disappears. By compression of the bulb the column of mercury with its float is made to assume different heights. After the proper adjustment of the needle to the smoked paper has been made, bolometric curves are taken for the different pressures, the connection with the bulb being each time cut off. A slow movement of the paper is sufficient, because the method is concerned with the height, and not the form, of the curve. Hence a large

series of pulse-waves can be recorded on one line of a paper 6 cm. wide, and by moving the smoked paper back and forth curves for the different pressure heights may be traced one below the other. With such long tracings we can often note distinct Traube's waves corresponding to a periodic increase and decrease in the mercury excursions. In such instances the cardiac work, and not alone the blood-pressure, is evidently increased and decreased. The zero pressure line is given the position of the writing point when the manometer is at rest; the hight of pressure corresponding to each curve can then be easily determined by measuring in millimeters the distance from the zero line to a horizontal drawn later to intersect the points half-way between the tip and foot of the waves. Since the manometer is constructed with two limbs, this distance must be doubled. It is self-evident that this doubling only applies to measuring the mean position of the mercury, not the height of an individual wave. For reckoning the work done by the pulse under observation we select that curve which shows the greatest excursions, since at that moment the transmission of energy was most nearly complete.

It remains to consider the clinical value of the results obtained by sphygmobolometry. It is obvious that we are computing not the entire energy of the heart, but only that fraction which acts upon the portion of the arm stump obstructed by the Esmarch bandage and corresponding to the surface of the cuff. The muscular development of the arm would naturally affect this fraction to some extent. But in healthy individuals the differences between the results are found to be so small, and in pathologic conditions of the heart so great, that the author believes it possible to consider the result found as an approximately constant fraction

of the total work done by the heart in each case. Conclusions may, therefore, be drawn concerning the entire amount of cardiac work—a result impossible by any previous method. The objection may be made that we are measuring simply the *wave energy* in the artery, and taking no account of the *current energy.* The author, however, believes such a criticism to be entirely unwarranted. For, if we measure the wave energy in a peripherally closed artery, we are really measuring the entire energy from the heart exhibited at that point, because the current at the periphery through the capillaries, which determines the amount of the circulation, is entirely due to this wave energy. The pressure in the capillaries arises wholly from the change of kinetic wave energy into potential pressure energy. This is illustrated by a fact easily shown by the sphygmograph. If even a single pulse be lost, the minimal pressure, i.e., the base of the curve, instantly falls. The arterial pulse is therefore to be conceived as a kind of peripheral heart, working not automatically, to be sure, but by its elasticity, or, to a certain extent, by its resiliency. This conception of the pulse is set forth and defended in the author's work on the absolute sphygmogram. He considers it a most important one, and exceedingly fruitful in any study of the dynamics of the circulation. It at once makes clear the significance of sphygmobolometry. If one assume, then, that by the sphygmobolometer he measures an approximately constant fraction of the entire heart energy (its exact value depending upon the constant of the instrument), it is possible to get at least approximately relative values for the active circulation.

It should be added that this procedure corresponds to the old-fashioned method of feeling the pulse, which sought to estimate the force of the pulse-wave rather

than the height of the blood-pressure. This method has been entirely neglected of late on account of the disproportionate modern insistence on the static conception of the circulation, and yet this method of feeling the pulse is perhaps the most practically useful one, since it focuses the attention on the work done by the pulse-wave without regard to the blood-pressure. It *appreciates* the pulse energy, which the more exact method of bolometry records and measures. This method measures, not the amount of work which the heart is capable of performing under increased demand, but the amount which it actually performs at a given moment, e.g., the performance of the heart and the size of the circulation in a healthy individual at rest may appear very small; and yet this does not preclude such a heart's capacity for a very much greater task under the stimulus of vigorous muscular work. Hence in a given case the functional examination may have to be supplemented in this respect by noting the alteration of the sphygmobologram after making an increased demand on the heart, e.g., lifting a weight with the free arm. With a heart capable potentially the work (W) (measured sphygmobolometrically) increases materially; with serious cardiac insufficiency, on the contrary, this work does not increase and may even decrease.

Sahli has more recently devised a simplified pocket sphygmobolometer which he demonstrated at the XVIIth International Congress of Medicine at London in 1913, and which is described by N. B. Potter in the Journal of the American Medical Association for April 9, 1913. In this the graphic method of recording the maximal excursions is replaced by a visual method with a kerosene index in a blind compressed air manometer, connected with the recording manometer, and direct

compression of the radial artery by a small rubber bag and graduated air syringe is substituted for the circular compression of the brachial artery. As his original apparatus was rather cumbersome this simplified instrument will bespeak a wider usage of this important method of testing cardiac function.

4. Sphygmobolography

At the XVIIth International Congress of Medicine Sahli described a method for using Jaquet's sphygmograph for measuring the energy or working power of the pulse, which he termed sphygmobolography. His description is as follows: [22]

The possibility of this results from the following simple consideration. The mechanical effect of a force is known to be calculated as the product of force (or weight) and distance. In sphygmography the spring plays the part of the weight, whereas the excursion of the recording point represents the distance. So by multiplication of these two values expressed in grammes and centimeters we get the mechanical effect or the energy of a given sphygmogram in gramme-centimeters.

It is true that there exists the theoretical difficulty that the weight or force changes continually during the taking of a sphygmogram, because by the progressive elevation of the sphygmogram the tension of the spring increases. However, I was able to prove that in Jaquet's new sphygmograph the modulus of elasticity of the spring is nearly constant, so that the increase of the tension of the spring is nearly proportional to the excursion and rises in the form of a straight line. Hence for the calculation of the mechan-

[22] Sahli, H., in Transactions of XXVIIth International Congress of Medicine, London, 1913.

ical effect of the pulse we can take as the force (or weight) the arithmetical mean of the initial and the terminal tension of the spring. Thus if we know the amount of the spring-tension at the foot and at the summit of the sphygmogram in absolute measure (in grammes) the sphygmobolometric utilization of the sphygmogram (that is what I call sphygmobolography) presents no kind of difficulty.

To carry out the determination easily I have supplied Jaquet's sphygmograph with a little modification which can be adapted easily. This modification consists essentially in the adaptation of a recorder of abscissæ. This is composed of nine little wheels pressing the blackened strip of paper against the cylinder of the moving mechanism. The distances of these abscissæ are chosen so as to correspond to an excursion of the recording point of 0.005 cm.

These abscissæ have a double purpose. They serve to calibrate the tension of the spring for every level of the sphygmogram in absolute measure (grammes), and at the same time to show directly on the sphygmogram the travel of the recording point in centimeters. The maker adds to every instrument thus modified a calibrating table, marking for every position of the tension mechanism and for every abscissa the spring-pressure in absolute measure, that is in grammes.

So to calculate any given sphygmogram sphygmobolometrically we have only to measure the excursion of the recording point by the height of the sphygmogram with the aid of the calibrated abscissæ and to take from the said table the tension in grammes for the abscissa half-way between the foot and the summit of the sphygmogram. The multiplication of the two values will give us the amount of the energy or mechanical effect of the pulse.

Naturally one must bear in mind that just as in pneumatic sphygmobolometry, the optimum transmission of the pulse-energy to the recording apparatus is only obtained by one certain optimal counter-pressure on the artery. Thus for obtaining comparable values it is necessary in each case to take on one strip of paper the whole series of sphygmograms with all the different spring-pressures which give curves, and to choose that curve which gives, on calculation, the greatest energy value.

Example: For three successively taken sphygmograms I obtained the values:

1		2		3	
Tension	= 135.5 gm.	Tension	= 142 gm.	Tension	= 168 gm.
Excursion	= 0.0125 cm.	Excursion	= 0.015 cm.	Excursion	= 0.01 cm.
Energy	= 1.7 g. cm.	Energy	= 2.13 g. cm.	Energy	= 1.68 g. cm.

Thus the real energy would be 2.13 g. cm.

To apply this method to functional testing of the heart, it must of course be employed before and after exercise, the energy in the latter case being greater in a healthy heart, and showing no increase or a decrease proportional to the functional damage in an unsound heart.

5. *Energometry—Dynamic Diagrams*

Christen has advocated the dynamic diagnosis of the pulse, along lines similar to, though differing somewhat from, those of Sahli. He gives a detailed discussion of his methods, with elaborate mathematical proofs in a recent monograph.[23] The following account is taken from a simplified exposition which he prepared for the general practitioner:[24]

[23] Christen, Th. Die dynamische pulsuntersuchung, Leipzig, 1914.
[24] Christen, Th. Dynamic diagrams of the pulse. International Clinics, Philadelphia, 1911.

The Hypothesis.—The old-fashioned palpation of the pulse tells us much more about the condition of the circulation than does the sphygmogram, in spite of its seemingly higher scientific character. In the clinical sphygmogram there is no exact relation between the ordinates and the pressures. We do not even know where to trace the level of the pressure zero. Even if there were such a relation, sphygmography would still not be a dynamic method, as in dynamics we do not have to study only the temporal variations of the forces, but essentially the effect of these forces. These two statements sufficiently explain the failure of the clinical sphygmogram. There are two dynamic diagrams of the pulse that may be determined on a mathematically exact manner. They are the graphic expression of clinical experiments based upon the following two questions:

a. What is the systolic increase of volume of the arteries covered by a pneumatic cuff at a given pressure?

b. What is the amount of mechanical energy required for this movement? These dynamic diagrams, called stowing curves, are characteristic of the behavior of the pulse against a stowing pressure. They depend on the volume of air within the cuff as little as they do on the elasticity of the cuff and the soft parts. They depend only on the breadth of the cuff. We therefore have to compare stowing curves derived from experiments with cuffs of the same breadth. The exclusive arguing with the notion of pressure and its temporal variations will never be able to exhaust the question of diagnosis of the pulse, for the mechanics of the pulse contain essentially dynamic problems, more than any other province of physiology. The stowing curves (dynamic diagrams) replace the palpation of

the pulse by an exact method, because they answer the same questions that does palpation, informing us about the filling and the intensity of the pulse. They are to inaugurate a new epoch of clinical research, because for the first time there has been established here a correct dynamical principle.

The Method.—The energometer consists of a close fitting cuff, 9 cm. wide, connected with an inflating bulb, a special manometer and a graduated air syringe. The cuff is applied to the patient's arm or the calf of the leg. The latter is preferred, owing to the possibility of greater muscular relaxation and absence of disagreeable sensations.

In order to find out the value of the systolic increase of volume, i.e., the volume of blood, the pushing forth of which against the pressure of the cuff produces the oscillations observed on the manometer, we make use of a syringe, whose piston has to be pushed in up to the point where the oscillations have been displaced about its own amplitude.

Example: We observe on the manometer oscillations between the limits 170 and 176. Pushing in the piston of the syringe we increase the pressure within the cuff, elevating both limits of these oscillations. We do this in such a manner as to just reach the point where 176 has become the lower limit of the oscillations, having been heretofore its upper limit. Thus we are sure that the volume of the piston, which is read off on its own scale, must be that incompressible volume which brought under the manchette increases the pressure within it from 170 to 176. Two incompressible volumes, which, forced into the same gas-room, produce the same increase of pressure within it, must be equal. Therefore, the volume read off on the piston must be equal to the systolic increase of volume of the

artery (or arteries) that is covered by the inflated manchette.

Suppose that we read a volume of 0.7 centimeter, then we know that the mechanical energy required for the same increase of volume—the mean pressure being 173 gr./cm—must have the value:

$$173 \frac{\text{gr.}}{\text{cm.}} \times 0.7 \text{ cm.} = 121 \text{ gr. cm.}$$

In this way we find for every pressure (P) a volume (V) and an energy (E), the relation between which is

$$E = P V$$

Repeating this experiment at different pressures we get a series of pressure volumes and energies, which we arrange in the following manner:

Example:

Pressure	Volume	Energy
80	0.1	8
120	0.3	36
140	0.55	77
165	0.9	149
190	1.2	228
210	1.2	252
225	0.9	203
240	0.4	96
250	0.2	50

The Diagrams.—In order to give a clearer idea of the relation between these quantities, viz., pressure, volume, and energy, we plot two curves, which represent the volumes or the energies as functions of the pressure. Therefore, in our graphic method we have to plot the pressure as abscissæ and the volumes or the energies as ordinates.

Functional Diagnosis.—The study of the blood circulation under different conditions, as after any therapeutic procedure whatsoever, medicines, cures, or after a given dosage of labor, is done in the most exact way

by the dynamic diagram. Here we are able to show now a rise or fall of the summit, now a displacing towards a higher or a lower pressure, as in a sharpening or flattening of the curves.

The methods of Sahli and of Christen, introducing as they do the underlying principles of dynamics into the study of the pulse and the systolic output of the heart, are of great promise, and have already shown their value in clinical work when applied to functional testing of the efficiency of the cardiac muscle.

6. *Röntgenoscopy and Röntgenography as Indices of Cardiac Function*

The form, position and movements of the heart as a whole and its different chambers, also the great vessels at its base, can be very successfully examined by the X-ray. For several reasons we need only touch upon this interesting and remarkable method of cardiac examination. In the first place, the application of the X-ray to the study of the heart concerns more particularly the examination of the organ from a diagnostic and anatomical point of view. From this standpoint it constitutes a valuable addition to the older methods of heart exploration. It cannot be said, however, that a Röntgen ray examination sheds much light upon the question of cardiac function. Its chief use is to denote changes in the shape of the organ, hypertrophy and dilatation of its cavities, aneurysm, pericardial effusions, etc. By orthodiagraphy, the position and topography of the heart can be accurately delineated. But neither ordinary Röntgenoscopy nor orthodiagraphy of the heart sheds much light upon the problem of estimating the exact efficiency of the cardiac function.

7. *Sphygmocardiography and Electrocardiography; Their Relation to Cardiac Functional Capacity*

In many text books the phrase, functional disease of the heart, is often used synonymously for pulse irregularity. The phrase, cardiac function, is used in an entirely different sense here. Cardiac function, so far as the present discussion is concerned, relates to the capacity of the heart to perform its work, with the adequate maintenance of its reserve. An irregularity of the cardiac rhythm does not necessarily mean any serious deterioration of function. For example, an individual may have a sinus arhythmia or an occasional premature contraction and possess an absolutely normal cardiac reserve.

On the other hand, the discovery of certain other types of irregular rhythm always indicates a serious disturbance of heart function. The presence of true heart block, for example, denotes a lesion of the conducting system and hence a deterioration of function. The same may be said of auricular fibrillation and to an even greater extent of pulsus alternans.

But the detection and identification of irregularities in the cardiac rhythm is a part of the general semiological investigation of that organ and while of great importance to the clinician who is examining a case for heart disease, does not properly come within the scope of an investigation into the methods of estimating cardiac function.

The study of cardiosphygmography and electrocardiography has undergone a tremendous development in recent years. The names of Marey, Franck, Gaskell, Engelmann, Wenkebach, Herring, MacKenzie, Lewis, Erlanger, His and many others are prominently identified with the former and those of Waller, Ein-

thoven, Kraus, Nicolai, Edelmann and others with the latter.

The recognition of nearly all the varieties of arhythmia may be determined by the skilled clinician without the use of any technical apparatus. Unfortunately, this is not always the case and there are types of irregularity of the heart rhythm which can only be positively recognized by the use of some form of instrumental registration. Pulsus alternans is the best example of this fact. This variety of arhythmia cannot be recognized with certainty without pulse tracings. Its importance from a prognostic standpoint, as Lewis points out, is extremely great. This fact alone will always make the polygraphic study of the pulse a matter of necessity in all cases in which there is a suspicion that pulsus alternans may be present.

The ordinary methods of examining the heart, employed in clinical diagnosis, are exceedingly well adapted to disclose diseases of the organ. By inspection, palpation, percussion and auscultation, properly performed, not only can it be determined that disease of the heart is present, but the precise location and often the nature of the lesion can be made out. As Cabot has well expressed it, "we are very well satisfied with the ordinary methods of examination, when we find something such as valvular disease, obstructions, accumulations and degenerations. But in many cases in which we fear that the heart is diseased and its functional power diminished, the ordinary methods of investigation do not show anything. Even the more technical and refined instrumental methods are negative only too often in such cases. The heart has passed a good physical examination and yet may be insufficient. We desire to know what the heart can do, what is the condition of its reserve. The necessity of supplemental methods

becomes manifest. This is the proper field for experimental inquiry into the heart function. It is here that the functional tests become especially useful. We give the heart some work to do and see how it reacts, how fast it tires, how slowly it recuperates. We subject the patient to extra effort and note the general symptoms produced, particularly dyspnœa. We proceed to a careful analysis of the history of our patient with respect to his subjective reaction to all of his environment. Already we are working with problems of cardiac function in a fundamental manner."

GENERAL CONCLUSIONS AS TO TESTS FOR CARDIAC FUNCTION

Hirschfelder, speaking upon the importance of functional tests or studies in borderland cases between functional sufficiency and cardiac failure, emphasizes the importance of careful observation and says: "It must be admitted that in order to be decisive, all tests have to be pushed to a point at which the appearance, sensations and signs of the patient are in themselves perfectly characteristic of cardiac insufficiency and at which, for diagnostic purposes, a little common sense observation is at least as unambiguous as observation with elaborate apparatus. This does not mean that exercise tests are unimportant. On the contrary, they are of the greatest value and no change in the patient's mode of living during convalescence or during after life should be undertaken without them.

"But their importance depends more upon the care with which the physician watches the general appearance and condition of the patient, the rapidity with which he recovers from the exercise, his general condition and whether nervousness, irritability, cough or in-

somnia have set in during the 24 hours following it, than in the numerical changes which occur at the moment of exercise. The symptoms to be looked for as evidence of overwork are well known. These are subtler manifestations resulting from smaller changes than may be detected by even the most refined observation by mechanical methods and which are less easily masked by ambiguities.

"Moreover, it must be realized that any one form of exercise furnishes data which may depend as much upon the condition of the skeletal muscles as upon the heart. The blacksmith with a diseased heart may be able to do more work than the bookkeeper with neurasthenia and yet under the conditions in which he lives even if not under the strength test arranged for the average man, the blacksmith's heart may be failing. In diagnosis, prognosis and therapy the testing of functional insufficiency is a matter of sociology as well as physiology. The important question is not what the person can do in a gymnasium, but what he can do and what he cannot do in everyday life. Each man must be fit for his own mode of life or must be made to change it. His cardiac power must be studied with reference to that mode of life rather than with reference to a rigid scheme." [25]

[25] Diseases of Heart and Aorta, Phila., 1913, 199.

CHAPTER V

THE DUCTLESS GLANDS AND VEGETATIVE NERVOUS SYSTEM

GENERAL CONSIDERATIONS

THE clinical examination of function of the endocrinous glands is a subject capable of great future growth. Only the first steps have so far been taken in developing this mine of hidden riches. To the physiologist, pathologist and clinician the subject offers a fertile and tempting field of investigation.

In the following account of the functional diagnosis of the endocrinopathies but little can be given of the enormous mass of experimental material, and, as Barker [1] has said, "the greater mass of theories" propounded, concerning the physiology, semiology, pathology and interrelations of the glands of internal secretion. These subjects with complete bibliographic and historic references can be found in the classical works of Biedl,[2] Vincent,[3] Falta,[4] Levi-Rothschild,[5] Paton,[6] Sajous [7] and others.

In these great works little or nothing can be found

[1] Southern Med. Jour., 1914, VII, 1.

[2] Internal Secretary Organs, London, 1912, Bale Danielsson (Trans. from German).

[3] Ductless Glands, London, 1912, Arnold.

[4] Erkrankungen der Blutdrüsen, Wien, 1913.

[5] Endocrinologie, Paris, 1911, Dion.

[6] Internal Secretions, Phila., 1911, Davis.

[7] Regulators of Metabolism, London, 1913, Macmillan.

concerning the important question of functional diagnosis. The material that has been evolved concerning the investigation of function of the ductless glands or glands of internal secretion is not only scant but scattered widely in the literature from whence so far as we know it has never been gathered. Not a single article devoted to the general question of the functional diagnosis of the endocrine glands exists in the whole medical literature. In isolated instances where the subject of functional diagnosis is mentioned, upon investigation it is found that the question is treated upon an almost purely semiological basis. The semiological method of diagnosis of diseases of the ductless glands has therefore reached a higher degree of development than the functional method, among clinicians up to the present time. Notwithstanding all this, it is admittedly true that the clinician is much in need of functional tests, to enable him to discover the various endocrinopathies in their latent stages, or, as the French say, in the *forme fruste*, when the symptomatic picture may be incomplete or confusing. Therefore, while at the present time it cannot be said that satisfactory chemical or biological functional tests have been discovered, capable of disclosing with certainty the existence of latent disease of the thyroid, parathyroid, thymus, pituitary or adrenal organs, nevertheless some advance has been made in this direction and the great need for such tests, in this important tho subtle field of clinical medicine, will always constitute a sufficient inspiration for further discovery.

As will be developed, the principal tests which have been devised up to the present time with the object of testing endocrinous function, relate to the thyroid gland and particularly with that aspect of thyroidopathy which is connected with an increased activity of the gland. Function testing of the adrenals has received

some attention and development. The other ductless glands remain so far a *terra incognita* to the functional method. When we stop to consider how much remains to be known concerning the functions and interrelations of the glands of internal secretions, the fact will not be surprising that the subject of functional diagnosis of their diseases has not received a greater development.

Regarding the ductless glands as a whole, we cannot fail to be impressed with their intimate relation to the processes of body metabolism. The organs of internal secretion or ductless glands (*blutdrüsen*) are important regulators of metabolic processes. It is agreed that the pancreas normally inhibits carbohydrate metabolism and that on the other hand the thyroid and suprarenals normally increase carbohydrate metabolism. The thyroid has an important effect upon proteid metabolism not shared by the other glands. Increased function of the thyroid is accompanied by increased proteid metabolism while hypofunction of the thyroid produces a diminution of proteid exchanges. The parathyroids and thymus are intimately concerned with normal calcium metabolism but their exact relation to this process is unknown. The gas exchanges of the organism are also fundamentally controlled by the ductless glands. In hyperthyreosis there is an increase, and in myxedema a decrease, of the oxygen absorption and CO_2 exchange.

These facts have formed the basis of certain experimental methods of determining the functional capacity of the ductless glands and were it not for the fact that the performance of metabolic experiments is so complex and requires so extensive an instrumental equipment, the examination of these processes as aids to the functional diagnosis of the endocrinopathies would have a much wider application.

I. THE THYROID GLAND

1. *Tests of Functional Activity*

Without entering debatable ground, it may be confidently asserted that there are certain facts regarding the physiology and pathology of the thyroid gland which are universally admitted. It is clear, for instance, that the thyroid is of great importance in the economy of the human organism, and that certain lesions of this gland give rise to symptoms which when they are outspoken may be definitely correlated with thyroid disease.

However, despite all the work, experimental and clinical, which has been done upon this gland, there is even now no absolute unanimity of opinion of the precise function or functions of the thyroid. The thyroid function is probably not simple but complex. As in the case of the other ductless glands, many physicians feel convinced that there is an antitoxic function of the thyroid, which consists in collecting exogenous iodine and in some mysterious and unknown way neutralizing certain hypothetical products of intermediary metabolism. Naturally this idea is a pure speculation and has been arrived at by indirect reasoning. The most usually accepted theory of thyroid function is, however, that the thyroid manufactures an internal secretion, possibly an iodine proteid, which is essential to the proper growth and normal metabolism of the entire body. Baumann [8] made an epochal discovery in 1895 when he showed that the thyroid gland contains iodine. Although speculation and investigation have since been rife in respect to this discovery, its precise meaning is yet unknown.

The vast amount of work which has been done upon

[8] Zeitsch. f. physiol. Chem., 1895, XXI, 319.

experimental extirpation of the thyroid can only be mentioned. If the thyroids are removed from young animals, growth is retarded. Total extirpation of the thyroids in human beings is well known to be followed by serious symptoms, the so-called cachexia strumipriva. If the individual is young there will be retarded growth, faulty ossification, mental and metabolic enfeeblement. Similar symptoms are seen in children with congenital thyroid aplasia. In human beings who are deprived of their thyroids, a phenomenon appears which is not seen in lower animals, namely myxedema.

Spontaneous myxedema also occurs in the adult human being as a result of thyroid insufficiency—the so-called Gull's [9] disease. Murray [10] discovered that the administration of thyroid extract will eliminate the symptoms of myxedema.

If thyroid substance be fed to normal animals, symptoms will develop which are similar to those that occur in Graves' or Basedow's disease in the human subject and are supposed to be due to a hyperthyreosis or hyperthyroidization—in other words, an increase of function of the thyroid.

This brings us to the important induction that in the human subject we may have two different or rather two opposite pathological states to consider in respect to the thyroid gland and its functions, namely A. a hyperthyreosis and B. a hypothyreosis. In the well developed state these two symptom groups constitute definite and tangible clinical syndromes.

2. *Hyperfunction of the Thyroid Gland*

To the syndrome of hyperthyreosis, the name of Graves' or Basedow's disease is attached. The symp-

[9] Trans. Clin. Soc., London, 1874, VII, 180.
[10] Brit. Med. Jour., 1891, 796.

toms of hyperthyreosis may be placed in the following categories: 1, enlargement of the gland; 2, signs of heightened excitability of the vegetative or sympathetic nervous system; 3, signs of secondary or concomitant disturbances in other endocrinous glands; 4, symptoms of profound disturbance, usually of excess metabolism; 5, a variety of disorders of the central nervous system; 6, a peculiar picture in the blood (leucopenia with lymphocytosis).

Naturally in this review of functional diagnosis we cannot go into a detailed account of the semiological data which might be collected under each of the above headings. It is interesting to observe that the symptoms of Graves' disease which come under the category of the vegetative nervous system involve both the autonomic and sympathetic portions of that system. The autonomic and sympathetic innervations are both involved to a certain extent in every case, but in one, the former, and in another case, the latter, system will be predominantly affected. The eye, heart, blood vessels, skin, digestive, respiratory and urogenital apparatus are all supplied with innervations of both kinds, usually reciprocally or antagonistically, and consequently in Graves' disease there are symptoms referable to disturbances in sympathetic or autonomic innervations in several or in all these different organs. We shall not enumerate all the various symptoms of Graves' disease, since such an enumeration will be readily found in books or articles dealing with the semiology of this condition. Whenever a sufficient number of these symptoms can be found in a given case the diagnosis of Basedow's disease can be readily made and a quantitative idea of the severity of the case may be gained by the actual number or the seriousness of the symptoms. In other words, when the classical semiology of Graves' disease is pres-

ent, naturally no functional tests will be needed.

A striking phenomenon of Graves' disease is the acceleration of all the metabolic processes. This acceleration includes the total combustion in calories, the protein, carbohydrate, fat and mineral metabolism. In states of hypothyroidism the opposite condition of retardation of metabolic processes occurs.

Functional tests have therefore been proposed as a criterion of the existence of states of hyper- or hypothyreosis on the basis of increased or diminished oxygen consumption and protein, carbohydrate, fat and mineral metabolism.

As simpler methods of determining the activity of these various processes are developed we may hope that this kind of investigation will gradually enter more and more into the diagnostic armamentarium of the clinician who is interested in measuring the functional integrity of the thyroid. But as was said before, the technical difficulties which have so far usually surrounded the methods of determining and measuring the various processes of metabolism have prevented their general introduction into clinical medicine.

The symptoms of Graves' disease which are referable to concomitant or reciprocal disturbance of function of the other endocrinous glands have contributed somewhat to the diagnosis of hyperthyreosis from a semiological standpoint. This circumstance we shall not attempt to develop. But from the standpoint of functional diagnosis of the thyreopathies, these disturbances acquire a considerable importance since they open the way, though indirectly, to the development of means for testing the functional activity of the thyroid. It is quite generally held that the thyroid and the pancreas mutually inhibit one another's activity. The pancreas and chromaffin system (adrenals) are likewise

mutually inhibitory. The thyroid and the adrenals appear, however, to reciprocally favor each other's activity, that is, an under function of one leads to an under function of the other, while an over function of the one will lead to an over function of the other. This is the teaching of the present Vienna school of endocrinologists represented particularly by Eppinger, Hess, Falta, Rudinger, and others. According to the teaching of this school, a hyperthyroid function will be accompanied by an insufficiency of pancreatic function (internal secretion) and by an increased activity of the chromaffin or adrenal system. Hypothyroidism, on the contrary, will be followed by over function of the internal secretion of the pancreas and diminution of adrenal functional activity.

On the basis of these hypotheses, certain tests have been devised to disclose a hyper function of the thyroid gland. One of these, the so-called adrenalin-mydriasis test of Loewi, is used to disclose on the one hand an insufficiency of the internal secretion of the pancreas and on the other a hyperthyreosis. So far as its application to the investigation of pancreatic insufficiency is concerned, the test has already been described (*v. s.*) With reference to the second application, namely that of disclosing a hyperthyreosis, details will be later given. Tests founded upon the existence of a glycosuria, either spontaneous or following the injection of adrenalin, will be considered under the heading Adrenal Glands.

The diagnosis of hyperthyroidism, or Graves' disease, is easy in typical cases. The enlarged thyroid, the tachycardia, the disturbances of the sympathetic nervous system, the tremors, the mental state, the accelerated metabolism and the blood findings make a definite and indubitable diagnostic picture.

There are, however, many cases which elude the clinician because they are atypical. To these latent or atypical cases the French have given the expressive title of *formes frustes.* Barker [11] has warned us very properly that any one of eight different symptoms should make the clinician suspicious of Graves' disease. The symptoms are: (1) persistent tachycardia (pulse above 85); (2) rapid emaciation without apparent cause; (3) excessive sweating; (4) persistent watery diarrhœa; (5) neurasthenic and psychasthenic states; (6) outspoken lymphocytosis; (7) fine tremors of the fingers; (8) one or more of the usual ocular symptoms of the disease, namely *protrusio bulbi* or positive Dalrymple,[12] von Graefe,[13] Moebius',[14] Stellwag,[15] Jellinek [16] and Rosenbach [17] signs.

Suppose, however, one of these suspicious signs be present. How shall it be determined whether or not there is actually present a condition of hyperthyroidism? It is here that a satisfactory functional test would be invaluable. Frederich Müller first called attention to its necessity under such circumstances. Several functional tests are at present available for this purpose. Unfortunately, they have not been found entirely adequate. Nevertheless, they are of a sufficient degree of assistance to warrant their retention by the clinician, especially as they form an important nucleus upon which future investigators may build new theories and points of departure for renewed attempts at exploration. Some of them have not been sufficiently developed as yet to allow a final opinion to be formed.

The tests of hyperfunctional activity of the thyroid gland are as follows:

[11] Southern Med. Jour., 1914, vii, 1.

[12] Widened eye slit; [13] lagging upper lid; [14] insufficient convergence; [15] infrequent incomplete winking; [16] pigmented eyelids; [17] tremor of closed lids.

1. Hypophysis Test of Claude, Baudouin, and Porak.
2. The Adrenalin Mydriasis Test of Loewi.
3. Induction of Experimental or Artificial Hyperthyroidism as a Functional Test.
4. The Aceto-Nitril Test of Reid Hunt.
5. Metabolic Studies in the Functional Diagnosis of Hyperthyroidism.
6. The Complement Fixation Reaction as Functional Test of Hyperthyroidism.
7. The Specific Ferment Reaction of Abderhalden, as Functional Test of Hyperthyroidism.

1. The Hypophysis-extract Test for Hyperthyreosis. Claude, Baudouin, Porak Test [18].—Recently Claude, Baudouin and Porak have published some interesting researches upon the use of extract of the posterior lobe of the hypophysis, in disclosing the presence of latent hyperthyroidism.

In their experiments they made use of a hypophyseal extract of posterior lobe of such strength that 1 c.c. of the substance to be injected was equivalent to ½ of a posterior lobe of a beef's hypophysis. This they say corresponds to .05 of hypophysis powder.

This extract was obtained by the action of alcohol at 70° upon the hypophysis powder, dried and freed from fat. The alcohol is evaporated and the residue redissolved in normal salt solution. They also employed occasionally a watery extract, obtaining results with the latter which were practically similar to those obtained from the former.

They found that subcutaneous injections of both watery and alcoholic extracts of hypophysis produced a marked reaction, pallor, glycosuria and diarrhœa, the

[18] Bull. et Mem. Soc. Méd. d. Hôp. de Par., 1914, XXX, No. 22, 1904.

greater effects being produced by the watery solution and accompanied by greater pain. The alcoholic extract was found to be less painful and the pain less lasting.

When a quantity of alcoholic extract corresponding to one whole lobe (beef) was injected, the authors noted complex effects, such as an action upon smooth muscle fiber, a cardiovascular effect and an action on nutrition. The diuretic effect was doubtful.

The action on nutrition was characterized by glycosuria, the cardiovascular effect consisted of acceleration of the heart. In these complex effects of the hypophysis there appears to be an excitation of the sympathetic system shown by the cutaneous vasoconstriction and the glycosuria. The accelerator fibers of the heart being likewise of sympathetic origin, one would naturally expect to find acceleration of the beat. One would also expect that with concomitant constriction of the peripheral vessels that the blood pressure would rise. This phenomenon did not appear. The blood pressure remained the same or was lowered. The authors attribute this to a depressing effect of hypophysis extract on the myocardium. They noted occasionally a galop rhythm following the injections. With normal individuals the authors noted acceleration of the pulse.

They then proceeded to experiment upon cases of Graves' disease. The patients were kept under observation free from emotional excitement until the normal pulse rate was accurately determined. Injections of plain salt solution were tried as controls.

Thirteen typical Basedowians were used in their experiments. With the exception of the cardiovascular effects the results of injections did not differ from those of normal cases. The symptoms, pallor, contraction of smooth muscle fiber of intestine

and uterus, and glycosuria, the latter fairly well marked, were noted in the case of Graves' disease. Alimentary glycosuria was more readily provoked after the injections than before, showing a diminution of the already lowered carbohydrate tolerance. The cardiovascular effects of the injections were highly significant. In normal subjects the pulse becomes accelerated. The acceleration commences two or three minutes after the injection. It reaches a maximum in 10 or 15 minutes. Then the frequency rapidly diminishes and in about 20 minutes the pulse is normal.

In the cases of Graves' disease, however, the results were found to be diametrically opposite. The pulse which is accelerated before the injection of hypophysis extract, becomes quickly slowed. In one of these cases the pulse dropped 42 beats. Usually the diminution in number of beats is much less, averaging 8 or 10. The maximum lowering is reached in about 2 minutes, sometimes 4 or 6 and rarely even 10. The bradycardia is usually ephemeral. Usually in 7 or 8 minutes the pulse becomes fast again. Sometimes it was found to return to the original number previous to the injection. In the majority of instances, however, it remains notably beneath this point.

It would appear as a result of these interesting experiments that extracts of the hypophysis, which normally produce tachycardia, bring about an opposite effect, namely, bradycardia in case of Graves' disease.

The authors believe that the extract of hypophysis contains principles which simultaneously excite the terminations of both sympathetic and vagus fibers. This is the only explanation of the complex effects of the substance, differing from those of adrenalin, which is a pure sympathetic stimulant. Adrenalin, when injected, causes both glycosuria and tachycardia, but it

does not produce, ordinarily, pallor of the skin, nor contraction of intestine and uterus, as does hypophysis. The pneumogastric is generally considered the nerve which produces intestinal peristalsis. Since hypophysis produces peristalsis, it must stimulate the 10th pair.

If now we consider the effects of hypophysis in Graves' disease, we may begin by admitting that in this condition there is a general state of erethism or hyperexcitation of the entire sympathetic and parasympathetic (vagus-autonomic) systems. That the sympathetic is excited is proved by the exophthalmus, glycosuria and tachycardia. On the contrary, the symptom diarrhœa which is so constant and characteristic a symptom in Basedow's disease is explained by Eppinger and Hess and others on the theory of a hypervagotonia.

When one injects into a Basedowian an extract of hypophysis, with the exception of the pulse rate, the effects are generally similar to those obtained upon the normal subject.

The heart slowing phenomenon, however, found by Claude, Baudouin and Porak, in Basedowians following injections of hypophysis extract remains to be accounted for. The authors believe that the slowing is due simply to stimulation of the vagus nerve or 10th pair. They think that hypophysis extract acts on the cardiac rhythm of the non-Basedowian by stimulating the accelerator sympathetic. In the Basedowian, however, there is already a tachycardia which is due to the continual hyperexcitation of the sympathetics. These nerves being already in a state of hyperexcitation do not react to hypophysis extract. The terminations of the vagus which are not excited, therefore, feel the full effect of the hypophysis stimulation and the heart is temporarily

slowed while the effect lasts.

Naturally, the authors of the "hypophysis test" mention the possibility of its use in the diagnosis of latent forms of Graves' disease. If future use of this test should corroborate the early work of Claude, Baudouin and Porak in this regard the "hypophysis test" will become an important adjunct to the functional diagnosis of the hyperthyreopathies.

With respect to its applicability to the diagnosis of the *forme fruste* or latent form of Graves' disease, the authors report two very instructive cases. In one a woman 27 years of age, of neuropathic taint, suffering from tachycardia, dysmenorrhœa and slight hand tremor without thyroid enlargement, the injection of 1 c.c. of hypophysis extract produced a slight increase in the rate from 120 to 126 beats. The test was therefore negative. In the second case, that of a nervous man 47 years old, with a tachycardia (100 to 105 pulsations) with slight exophthalmia and slight hand tremors, with no apparent thyroid enlargement, the injection of hypophysis extract slowed the pulse from 100 to 84 in four minutes. The test was, therefore, positive. The case was a true latent form of Graves' disease, that is, the syndrome of hyperexcitation of the sympathetic nervous system presented by the patient was truly connected with and due to a hyperfunctionation of the thyroid gland.

The authors likewise found, which may be mentioned for its scientific interest only, that in cases of paroxysmal tachycardia the test is negative, as would on a priori grounds be expected. In this condition the pathogenesis resides not in the sympathetic nervous system as a whole nor in any dysfunction of the thyroid nor any other endocrinopathy but in changes that have taken place in the cardiac musculature.

2. The Adrenalin Mydriasis Test of Loewi[19].—In 1907 Loewi found that in pancreatectomized animals the instillation of 1-1000 solution of adrenalin produced marked dilation of the pupil. In human beings with diabetes Loewi found the same effects. In 30-60 minutes a marked dilation occurred in diabetic cases. The application of this phenomenon to the detection of pancreatic insufficiency has already been mentioned.

Loewi also made a simultaneous observation that the instillation of 1-1000 solution of adrenalin into the conjunctional sac in cases of Basedow's disease, likewise resulted in dilation and proposed the method as a test for hyperfunction of the thyroid gland on the ground that the internal secretion of the thyroid and suprarenal are synergistic, both acting by stimulating the sympathetic nervous system. In cases of hyperthyroidism, the sympathetic nervous system is in a state of increased irritability, therefore the dilator fibers of the iris which are governed by sympathetic nerves respond with abnormal alacrity to the instillation of adrenalin. Loewi's findings were corroborated by Falta [20] and Zak.[21]

Eppinger, Falta and Rudinger [22] found an increased adrenalin mydriasis in dogs which had been fed with thyroid extract. In depancreatized and thyroidectomized animals the reaction was absent. Eppinger and Hess [23] also reported the test positive in Basedow's disease.

The interesting and extremely simple test of Loewi

[19] Wien. klin. Wchnschr., 20, 1907, 782; Archiv f. exper. Path. und Pharm., 59, 1908, 83.
[20] Wien. klin. Wchnschr., 20, 1907, 1559.
[21] Verhandl. d. 25 Kong. f. inner. Med., 1908, 392.
[22] Wien. klin. Wchnschr., 21, 1908, 241.
[23] Verhandl. des 26 Kong. f. inner. Med., 1909, 385.

has not been much discussed in the literature in recent years. It would be interesting to determine whether an increased susceptibility of the iris sympathetic as shown by mydriasis exists in cases of latent Graves' disease.

3. Test of Experimental Hyperthyroidism. Administration of Thyroid Extract, Iodine and Iodide of Potassium as a Means of Disclosing Functional Hyperactivity of the Thyroid.—Fr. v. Mueller, who criticized the metabolism tests for hyperthyroidism as being too complex, suggested the administration of iodine as a means of disclosing hyperthyreosis. But apparently Mueller only made the general suggestion and did not elaborate any specified technique. Since no one else has done so, it cannot be said that an "iodine test" exists for determining the presence of a latent hyperthyroidism. Patients with hyperthyreosis often show intolerance to iodine by developing emaciation and tachycardia, after its administration.

Many attempts to produce experimental thyroidism in animals by feeding thyroid substance have been made, and there seems to be great variation in the resistance of different genera to thyroid ingestion. When symptoms appear in healthy animals the most constant signs seem to be emaciation and diarrhœa (Carlson,[24] Ballet [25]).

Kraus and Friedenthal [26] found that the intravenous injection of thyroid juice in rabbits also produces enlargement of the palpebral fissure, projection of eyeballs and enlargement of the pupil. Other authors have succeeded in obtaining similar results.

[24] Prac. Am. Physiol. Soc., 1910-11, XXVII, p. XIII.
[25] Limousin Med., 1896, XX, 69.
[26] Berl. klin. Wchnschr., 1908, 1709.

Since the introduction of thyroid preparations into clinical medicine, artificially produced hyperthyroidism has been observed following their indiscriminate administration. The continued injection of thyroid extract is frequently followed by symptoms of intolerance such as subjective sensations of heat, perspiration, palpitation or tachycardia and occasionally glycosuria, all of which denote hyperthyroidism.

A few cases have been reported in which a typical Graves' disease syndrome has been produced by the administration of thyroid extract. The symptoms disappeared after a suspension of the treatment.

It is a well known fact that the administration of iodides over long periods to cases of goitre may produce symptoms of hyperthyroidism (Kocher).[27] To these cases the name iodine-Basedow has been given.

The actual administration of thyroid extract, iodine, and iodide of potassium to disclose a latent hyperthyroidism or Graves' disease is not to be recommended as a routine procedure. Most writers advise against the use of iodine, iodides or thyroid extract in any case where there are signs of emaciation (Krecke [28]).

Taking all the above facts into consideration, it will no doubt be admitted that there is little, if any, justification for the administration of either iodides, iodine or thyroid extract in cases of suspected Graves' syndrome with a view of thereby developing indubitable signs of the disease. It cannot be said to be justifiable under any circumstances to attempt to convert a latent or doubtful into an outspoken case of Graves' disease for purposes of diagnosis.

There appears to be among many medical men a

[27] Verhandl. d. Deutsch. Ges. f. Chir., Berl., 1910, 396.
[28] Münch. med. Wchnschr., 1911, LVIII, 1601 and 1676.

lack of appreciation of the dangers which are attached to the indiscriminate use of thyroid extract and some surgeons have stated that many cases of Graves' disease coming under their observation for operation give a history of previous ingestion of thyroid extract. It would certainly seem rational to assume that nothing but harm can come from such a practice.

These facts are, of course, well known and appreciated by a very large majority of medical men, and because of this knowledge no systematic attempt has ever been made to develop a test of experimental thyroidism. The use of thyroid extract, iodides, or iodine for such a purpose can only be mentioned to be condemned.

4. The Aceto-Nitril Test of Reid Hunt.—It was in the effort to develop a quick and satisfactory method for comparing the physiological activity of different thyroid preparations that Hunt [29] discovered the remarkable fact that mice when fed upon thyroids develop an increased resistance to aceto-nitril or methyl cyanide, CH.CN. This substance produces toxic effects chiefly through the slow liberation of hydrocyanic acid in the body. Since thyroid feeding does not alter the resistance of mice to hydrocyanic acid, it is probable that its action, so far as aceto-nitril is concerned, is exerted upon the processes by which the substance is decomposed in the organism.

Hunt found that when small amounts of thyroid are fed to mice for a few days these animals acquire a markedly increased resistance to aceto-nitril. This

[29] Amer. Jour. of Physiol., 1899, III; Proc. Soc. Exp. Biol., N. Y., 1905, Oct. 18; Jour. Biol. Chem., I, 33, Oct., 1905; Jour. Amer. Med. Assn., 1906, XLVII, 790; Hygien. Lab. Bull., No. 47, 1907.

is true for both white and gray mice, although most of his experiments were performed upon the former variety.

A mouse which had received thyroid in the form of cakes, recovered from 17 times the relative amount of aceto-nitril fatal to a control. Another mouse recovered from 16 times the relative dose fatal to controls. A third mouse recovered from 11 times, a fourth from 6 times and a fifth from 2½ times the fatal dose to controls.

Hunt suggested this reaction as a delicate test for thyroid substance.[30] He found no other substance with an effect upon the resistance of mice to aceto-nitril at all comparable to thyroid. The test is more delicate than any chemical test.

Hunt suggested in 1907 that this method is adapted to throw light on the question as to whether there is an excessive amount of thyroid secretion in the blood in cases of Graves' disease. He applied the test in three cases. In one of these the blood of the patient had a marked effect in increasing the resistance of mice to aceto-nitril, indicating thereby an excess of thyroid secretion. In a second case the results were doubtful and in a third case, negative.

Hunt suggested that the test might have some diagnostic value though he points out that it is not necessary to assume that in Graves' disease there is always an excess of thyroid secretion present in the blood at all times.

To carry out the method, he suggested that the best results might be obtained by administering to mice 1 or 2 c.c. of blood made up with meal in the form of cakes, for 9 or 10 days before testing with

[30] Jour. Amer. Med. Assn., 1907, XLIX, 240.

nitril. Controls are indispensable. One-fourth of a milligram of aceto-nitril per gram of body weight of mouse may be fatal to a normal animal in a few hours. Hunt used doses of a fraction of a milligram, one-fourth, to several milligrams, 1, 2, 4, in his experiments.

Hunt believed as a result of his researches that the activity of a given thyroid preparation or substance is parallel with its iodine content.

The test suggested by Reid Hunt has not been extensively developed. His findings were, however, substantially corroborated by several authors, among whom may be mentioned Trendelenburg in 1910,[31] Ghedeni in 1911.[32]

Before a final judgment as to the value of this procedure can be formed it will be necessary to determine the resistance of mice to aceto-nitril after feeding with the blood of patients with Graves' disease in a large series of cases. Also experiments should be done to determine the resistance of mice to aceto-nitril after feeding with normal blood, for perhaps the test is so delicate that even the amounts of thyroid secretions present under normal circumstances may be sufficient to increase the animal's resistance. Of course the variations in natural resistance of the animals both as regards species and seasons which have already been demonstrated as well as the possible variations in the blood content of thyroid substance even under normal circumstances may so complicate and obscure results that the test may be found impracticable.

There is, however, something extremely suggestive about this type of biological experimentation which makes it seem probable that some such test will be

[31] Biochemische Zeitschr., 1910.
[32] Wien. klin. Wchnschr., 1911, XXIV, 736.

discovered in the future of real value in the diagnosis of hyperthyreosis.

5. Metabolic Studies as Criteria of Hyperthyroidism.—In Graves' disease, as was above mentioned, the metabolic changes are increased. Biedl says they are so characteristic of the condition as to constitute an important diagnostic criterion. The metabolism of Graves' disease is accompanied by a pretty constant increase in the expenditure of energy.

The respiratory gas interchange shows an increase of 50%, sometimes 70% to 80% in the amount of oxygen consumed, according to Magnus-Levy,[33] Salomon,[34] and others.

The increased production of heat is usually accompanied by an augmented metabolism of albumen and fats. The assimilation of carbohydrates is diminished in Graves' disease and it is for this reason that alimentary glycosuria is readily produced.

Kraus was the first to suggest that determinations of the respiratory metabolism (increase of CO_2 and N), by the use of the Zuntz-Geppert apparatus may be useful in the functional diagnosis of latent or outspoken hyperthyroidism. Fr. v. Mueller, however, observed that the method is too complicated for practical work.

Studies of basal metabolism can be calculated by indirect calorimetry from the oxygen absorption and the respiratory quotient, using a Benedict unit apparatus (mouthpiece and spirometer). At least three ten-minute periods are run and the average taken for that day's basal metabolism.

[33] Berl. klin. Wchnschr., 1895; Zeit. f. klin. Med., 33, 1897; Noordem's Handbook d. Path. Stoffwechs., II, 352, 1907.
[34] Berl. klin. Wchnschr., 1904.

Means [35] has recently reported some studies of basal metabolism and its relation to body surface in obesity, myxedema and pituitary disease. He found a diminution of 27% below normal in myxedema.

The emaciation which occurs in many cases of Graves' disease naturally points to an increased katabolism. Kocher found reduction in weight in 88% of his cases. As much as 15 to 20 kgs. may be lost in a few months. The loss of weight is an early symptom. The basic cause for this loss in weight is the remarkable increase in the caloric production which occurs in Graves' disease. By using the Zuntz-Geppert apparatus many authors have made the demonstration of increased oxygen consumption and increased CO_2 elimination. Magnus-Levy and Salomon have already been mentioned.

Experiments have also been made in the Voit-Pettenkofer apparatus which give like results. The increase of caloric production as was before stated may reach as high as 70% or more above normal. In some cases (Magnus-Levy) the oxygen consumption has been found from over 5 to nearly 7 ccm. per kilogram of body weight. Salomon has shown that these metabolic disturbances occur and may be demonstrated in the latent cases.

It is to be hoped that the gradual simplification of methods for studying metabolism will lead to practical clinical results which will undoubtedly find a rich field of application in the functional diagnosis of thyroid diseases.

6. Application of the Principle of Complement Deviation to Functional Diagnosis of Hyperthyroidism.

[35] Proc. Soc. fr. Exp. Biol. and Med., 1914, XII, 1913.

Marinesco-Roseo Test.—Marinesco[36] in 1911 and Roseo[37] in 1912 appear to have been the first to suggest that in Graves' disease there is thrown out into the blood serum sufficient thyroid substance (antigen) to give rise to the formation of antibodies (amboceptors) in the patient's blood. They therefore proposed to test for the presence of these antibodies in the blood serum of suspected cases of Graves' disease by means of an antigen prepared from thyroid tissue removed at operation from an outspoken case of Graves' disease.

Both Marinesco and Roseo have studied the reaction of fixation of alexine (complement) in cases of Basedow disease and they believe that the positive results obtained proved the existence of true specific antibodies in the blood in this condition. This test may in future be found useful from the standpoint of functional diagnosis.

The principle of the complement fixation or deviation test depends, as is well known, upon the observation that the injection of the living organism with bodies of a proteid nature, cells, bacteria, organ extracts, etc., results in the formation by the organism of certain antagonistic bodies called antibodies. Perhaps these antibodies are ferments. The bodies that are inoculated in order to produce antibodies are called antigens. Some antibodies such as the agglutinins and precipitins act directly on the specific agent or antigen which produces them. Other antibodies, such as cytolysins and hemolysins, act only in the presence of a third body, which is always present in blood serum or tissue juices to which the name complement has been given. It is upon this state of facts that the now famous Was-

[36] Deutsch. Zeits. f. Nervenheilk., 1911, XLI, 268.
[37] Biochem e terap. sper., Milan, 1912-13, IV, 1.

sermann reaction is founded. The performance of the complement fixation test particularly in the diagnosis of syphilis has become a part of the routine work of almost every well equipped pathological clinical laboratory. Consequently there will be no difficulty in a well equipped hospital for the clinician to have complement fixation tests performed.

I have been unable to obtain the exact results of Roseo's work. Marinesco's [38] observations upon the reaction of fixation of complement included two series of experiments. In the first he used an aqueous extract of goitre from a classical case of Graves' disease as antigen and the serum of the same patient for antibodies and that of four other patients with the same disease. For controls he used the serum of normal persons. The fixation was complete in the first case whose goitre furnished the antigen, while in two other Basedowians there was incomplete hemolysis, and in a fourth the hemolysis was complete as in a normal control. An objection which may be urged against this first series as pointed out by Marinesco is that he did not have at his disposition an extract of normal thyroid. Later, with the assistance of Madame Papazol, he repeated his experiments, this time making an examination of 23 sera, and using different extracts from the thyroid gland of cases of Graves' disease, also from one goitrous thyroid and one normal gland.

The extracts were prepared in the usual way for ether extracts. Eight grams of Basedow goitre were triturated in a mortar and 100 grams of ether added, drop by drop. The mixture was put in a sterilized glass and placed in a shaker. After shaking and filtration, the mixture was kept for 48 hours in the thermo-

[38] Loco citato.

stat. After the evaporation of the ether a little carbolated water was added, 40 c.c. for 8 grams of substance. The extract was then again shaken and filtered through cloth. The prepared extract was kept on ice in a dark bottle. The alcoholic extract was similarly prepared.

In the two cases in which Marinesco made use of an autoextract of the thyroid from cases of Graves' disease, the prevention of hemolysis was complete. He found the same absolute prevention of hemolysis in six other cases of Graves' disease when he employed thyroid tissues obtained from other Basedowians. Aqueous, alcoholic and ethereal extracts appeared to act about the same, but sometimes the ether extract seemed more active.

In most cases of Graves' disease Marinesco obtained either a total absence of hemolysis or an incomplete or partial one. On the contrary, the serum of Graves' disease cases never fixed complement in the presence of normal thyroid body or extract of ordinary goitre. He got the same results when he used serum from normal persons and Basedowian antigen. In one case, however, Marinesco and Madame Papazol found that the serum of a syphilitic patient gave a partial hemolysis with ether and alcoholic extract of normal thyroid. Other authors have likewise found a more or less complete fixation with syphilitic serum in the presence of normal thyroid extract. Mueller, of Vienna, in a personal communication to Marinesco stated that in a case of Graves' disease he had noted a fixation of complement in the presence of alcoholic extract of heart.

Marinesco believes that his experiments tend to show the presence of an antigen in the thyroid gland of Basedowians and that the reactions of fixation which he

obtained are not simply due to an increase in the active substance (internal secretion) of the thyroid gland but to a change in the colloidal state of this substance, due to the harmful effects of a pathogenic agent. Marinesco calls attention to the difficulty in penetrating more deeply into the mechanism of his fixation reaction since authors in general are by no means in accord in explaining the mechanism of the fixation reaction discovered by Wassermann in the serum of syphilis. Some authors like Wassermann himself, seeing in the reaction the existence of true specific syphilitic antibodies, others on the contrary considering it as a non-specific physical chemical phenomenon.

The practical possibilities for functional diagnosis of his findings are believed by Marinesco to be worthy of mention.

For the Marinesco-Roseo test it will of course be necessary to secure thyroid gland tissue at the time of operation in a case of outspoken Graves' disease.

As to the clinical value of the test so little work has been done by investigators subsequent to the reports of Marinesco and Roseo that no definite opinion can be formed as to its value. This will become a matter for future investigation. It is to be hoped that the test will be carried out in a sufficient number of outspoken cases of Basedow's disease to determine in just what percentage it will be positive. If the findings are corroborated, the test should be applied to a series of cases of suspected latent hyperthyroidism. At the present time the final decision as to its value remains *sub judice.*

7. Specific Ferment Test of Abderhalden Applied to Functional Diagnosis of the Thyroid.—Lampe[39] has

[39] Münch. med. Wchnschr., 1913, 26.

attempted to apply the Abderhalden dialysis method to the study of the blood serum of patients with Graves' disease. He believes that in the blood serum of these patients ferments exist which are specific for thyroid tissue.

This method has never been applied extensively to the functional diagnosis of incipient Graves' disease since its introduction by Lampe. The technical difficulties attached to the carrying out of Abderhalden's method and the many conflicting results obtained in its general use have prevented it from becoming popular in clinical practice. Perhaps in the future when the method is simplified and its precise limitations defined, some practical results may be hoped for.

Lampe first demonstrated in 1913 that normal blood serum obtained from healthy individuals does not contain any ferments capable of splitting the tissues of any of the organs.

In the same year Lampe and Papazolu [40] examined the effects of the serum from cases of Graves' disease to determine the presence or absence of proteolytic ferments specific for thyroid tissue. Lampe thought that by the results of the dialysis method he might be enabled to throw some light upon the question as to whether in Basedow's disease there is a hyperthyroidism or a dysthyroidism. He does not mention the possibility of employing the test as an aid towards the functional diagnosis of the disease.

Lampe argued that if in Graves' disease there is an over-production of the normal thyroid secretion, therefore a negative result of the Abderhalden reaction (serum + thyroid gland) would be expected because in this case there would simply be the introduction into the blood of a purely native protein only in increased

[40] Münch. med. Wchnschr., 1913, 28.

amounts and consequently no development of ferments. If on the contrary, Graves' disease is a true dysthyroidism, if, in other words, the thyroid gland in Graves' disease pours into the blood a qualitatively altered proteid secretion, produced by the pathological changes in the gland, then this secretion acting as a foreign proteid would be expected to stimulate the production of protective ferments, and the Abderhalden reaction would be positive. Lampe hoped also to be able to throw some light by his method upon the rôle which the thymus plays in Graves' disease.

Lampe and Papazolu experimented with the serum from Basedow cases upon normal thyroid gland, exophthalmic goitre gland, cystic and parenchymatous goitre, normal thymus, Basedow thymus and several other organs and tissues as ovary, testicle, kidney, suprarenal, pancreas, etc. In their article they give the protocols of experiments upon the serum of twenty-five cases of exophthalmic goitre.

In all cases in which the serum from the Graves' disease cases was allowed to act upon exophthalmic goitre tissue, the tissue was digested. In very few cases only was the reaction positive when normal thyroid tissue was used. In four out of five of the cystic goitre products, in almost all thymus and ovarian tissues they found the reaction positive. With all other substrata, kidney, liver, pancreas, etc., the reaction was negative.

Lampe believes that his researches demonstrate that in Graves' disease there is a true dysthyroidism and not a simple hyperthyroidism.

Principle of the Abderhalden Method.—The basic principle underlying the now much discussed method of Abderhalden [41] is the fact that albumen, being a

[41] Defensive Ferments of Animal Organism, Abderhalden; tr. by Gavronsky and Lanchester, Lond. Bale. Co., 1914.

colloid does not diffuse through animal membranes, while, on the other hand, the peptones, which are the first products of its decomposition, are diffusible. If albumen is put in a dialysing tube and the latter placed in water no albumen appears in the surrounding fluid, even after a considerable lapse of time. If pepsin and HCl are added to the albumen solution peptones are formed and will appear in the dialysate. If it is desired to determine whether a liquid contains any proteolytic substance or ferments, the solution may be placed in a dialysing tube and peptone will appear in the surrounding media.

In this way blood serum, cerebrospinal fluid, lymph, extracts of organs, etc., may be tested.

The actual carrying out of the Abderhalden method is extremely difficult, so much so that the method cannot be used in the ordinary routine of clinical work. If the method is to be tried the individual who proposes to do so will find it advantageous to consult the little work of Abderhalden himself, which has recently been translated and to which reference has been given.

3. Hypofunction of the Thyroid Gland (Myxedematous States)

The best concrete example of the loss of function of the thyroid in human beings is met with in those cases in which the whole gland has been removed by operation for goitre.

Reverdin [42] in 1882 was the first to describe the results of goitre extirpation. In 1882 Kocher [43] published his classical report on the same condition. The

[42] Rev. Méd de le Suisse Rom., 1882, 539.
[43] Archiv f. klin. Chir., 1883, XXIX.

names operative myxedema or cachexia strumipriva were given to this condition. Inasmuch as the thyroid gland is never completely excised at the present day the subject has become of historical interest only.

What is of greater practical moment is the fact that symptoms somewhat similar to the cachexia strumipriva may spontaneously arise in adult human beings and give rise to the now well known but only too often overlooked syndrome of spontaneous or idiopathic myxedema of adults, Gull's disease. The symptoms are produced by retrogressive changes in the thyroid gland. Similarly there may be congenital states of hypothyroidism and infantile types, developing after birth, the so-called sporadic cretinism. Finally in some countries a condition known as endemic cretinism exists. All the above types of disease are associated with a diminution of function of the thyroid gland.

In all states of hypothyroidism the gland itself undergoes retrogressive changes. There are symptoms referable to the skin and subcutaneous tissues, the nervous system, metabolism, the bones, blood, etc. We shall not attempt to go into details in regard to the semiology of these interesting conditions.

The diagnosis of myxedematous states is easy in typical cases but even here many cases are overlooked by the practitioner. In latent cases, however, the *formes frustes*, the diagnosis may not be easy. Sometimes the edema is taken to mean Bright's disease. I have seen the nervous symptoms, speech difficulties and gait disturbance ascribed to chronic alcoholism.[44] Kocher calls the latent form of myxedema, thyropenia. All authors call attention to the great frequency with which it is overlooked.

[44] Jour. Am. Med. Assn., 1915, LXIV, 986.

The functional diagnosis of thyropenia is intimately bound up with the treatment for there is but one difficulty and that is to suspect the disease. Once suspected there is one infallible test, the therapeutic test.

Therapeutic Test for Lowered Functional Activity of the Thyroid Gland.—This consists in commencing the administration of thyroid extract. If the case is one of thyroid insufficiency the symptoms will magically disappear. If they are not entirely gone or improved in two weeks, the test is negative. The condition is not one of hypothyroidism.

Thyroid gland is best given in the form of tablets of the dried gland. The tablets contain $1\frac{1}{2}$ to 5 grains (.1–.3 gm.) of desiccated thyroid gland. Begin by administering a small dose after each meal or less often. The patient should lie down for twenty minutes after swallowing the tablet.

The dose is gradually increased until 6-10 tablets per day are given, care being taken not to produce rapid heart action, sweating, diarrhœa or nervousness, which are symptoms of intolerance.

From a therapeutic standpoint, which point we cannot discuss in this place, it is well known that the administration of thyroid gland in states of hypothyroidism must be kept up indefinitely, for so soon as the treatment is stopped, the symptoms will invariably recur.

II. THE PARATHYROID GLANDS

The first person to specifically describe the parathyroids was the Swedish anatomist, Sandstrom, in 1880. Gley practically rediscovered them in 1891.

Since the latter date a very considerable literature has risen upon these interesting structures.

Following the removal of two or more of the four parathyroid glands in the human subject, tetany comes on in from 2 to 5 days. All the special symptoms of tetany are present. Trousseau's, Erb's, Chvostek's and Hoffmann's signs, with irritability of the nerves of special sense and the sympathetic, irregular pulse, arterial spasm, angioneurotic edema, spasm of gastrointestinal tract, leucocytosis and disturbance of heat regulation may occur.

After incomplete extirpation or temporary injury of the glands, milder symptoms occur called tetanoid or subtetanic hypoparathyreosis (Halsted). Sometimes the symptoms are latent and come on sometime after injury or as a result of pregnancy, trauma or infection. In the well-known infantile tetany lesions of the parathyroids have been found.

Other convulsive diseases such as epilepsy, paralysis agitans, myoclonus, myotonia and myasthenia have been supposed to be due to disease of the parathyroids, but the exact facts in this direction are as yet unknown.

The exact relation between the thyroids and parathyroids is not known, some thinking that an antagonism, others that a synergism, exists between the two.

There is no method at present known of experimentally determining the functional activity of the parathyroid glands.

III. THE THYMUS GLAND

The function of the thymus is as yet not definitely known. It is assumed that inasmuch as the basic

structure of the thymus is that of lymphoid tissue in general, that there is a related function between the two, in other words, that lymphocytes and eosinophiles are formed in the gland.

There are, however, present in the thymus structure some epithelial elments, the so-called corpuscles of Hassal. What their function may be is quite unknown. Many believe that in the epithelial cells an internal secretion is elaborated which has to do with the development of the skeleton, nervous system, sexual apparatus and general metabolism.

There is a general belief that a reciprocal action exists between the thymus and testes, since castration delays the involution of the thymus while removal of the thymus causes rapid development of the testes.

The fullest development of the thymus is reached at the end of the second year of life. From this time on to puberty it gradually atrophies and in adults is represented only by a small mass of fibrous tissue and fat. Occasionally, however, the thymus gland persists or undergoes hypertrophy, producing symptoms of tracheal stenosis with attacks of laryngeal stridor or asthma, and sometimes there is sudden death, the so-called *mors thymica.* A condition known as the *status lymphaticus* may gradually develop in which there is more or less anemia, with lymphocytosis, together with rachitic and gastrointestinal symptoms.

No tests have so far been devised for determining the functional activity of the thymus. The diagnosis of its diseases is strictly semiological and in the diagnosis, radiography has recently been of considerable assistance.

IV. THE SUPRARENAL GLANDS

Our more intimate knowledge of the suprarenal glands appears to date from the year 1855, when Addison published his famous work upon the disease which bears his name, though they have been known since 1564, the date of their discovery by Eustachius.

A tremendous amount of work has been done upon the adrenals by investigators in the past half century. Much remains to be learned but certain facts appear to have been gained. These may be briefly stated as follows: disease of the glands resulting in their gradual atrophy or destruction gives rise to a train of symptoms characterized chiefly by pigmentation of the skin and extreme weakness, i. e., Addison's disease. Extirpation in animals of both adrenals is an extremely dangerous operation and according to most authorities leads infallibly to death. Extracts obtained from the medullary or central part of the organ are toxic when administered to animals, among the symptoms being glycosuria and arterial degenerations. The same extracts when injected intravenously produce a powerful constriction of the blood vessels with rise of the blood pressure due to stimulation of the sympathetic nervous system.

It is generally accepted by clinicians and pathologists that the adrenal medulla elaborates an internal secretion and that adrenin is the product of this secretion.

The exact function of the adrenal cortex is still unknown. The cortex contains a considerable quantity of lipoid and cholinogen substances, the presence of which has given rise to the hypothesis that neutralization of toxic substances is effected here.

In 1894 Oliver and Schafer noted that extracts of adrenal medulla produce a rise of blood pressure. In

1897 and 1898 Furth and Abel and in 1901 Takamine and Aldrich succeeded in gradually separating and finally isolating in pure form the active substance of adrenal medulla—adrenin.

From a clinical standpoint the functional activity of the suprarenal glands may be considered from two points of view. From the first the functional activity of the glands may be considered to be lowered and from the second it may be regarded as raised. To the first condition, the name hypoepinephria or hypoadrenalism has been given, and to the second hyperepinephria or hyperadrenalism.

In our brief discussion of the functional examination of the suprarenal glands this classification will be found most practical.

We may properly allude here again to the antagonism which is generally considered to exist between the function of the adrenals (chromaffin system) and the pancreas. It has already been stated that the adrenals and thyroid are functional synergists. The adrenal secretion inhibits carbohydrate catabolism and raises blood sugar, while the pancreas hormone facilitates carbohydrate catabolism and lowers blood sugar. The effect then of lowering the adrenal function is to raise that of the pancreas, namely, to facilitate carbohydrate catabolism and lower blood sugar to lead to hypoglycemia, oliguria and hence absence of glycosuria. The effect of raising the adrenal function will be to inhibit carbohydrate catabolism and to raise blood sugar, hence to lead to hyperglycemia, diuresis and glycosuria.

1. Hypofunction of the Suprarenal Glands

Several different clinical forms of hypoepinephria or lowered adrenal function have been described, but few

of them have been generally recognized by clinicians as separate morbid entities. The essential features of lowered adrenal function appear to be myasthenia and hypotension. The systolic blood pressure is usually below 100 mm. Other features justifying a suspicion of hypoepinephria are hyperesthesias, lumbar pains, headache, delirium, coma, digestive disturbances and sudden death without previous symptoms.

The chief clinical entity which is recognized as being accompanied by a true persistent hypoadrenalism is Addison's disease.

Addison's disease, first described by Thomas Addison in 1855, is a chronic condition usually appearing in the third or fourth decade of life. It is characterized clinically by pigmentation of the skin and mucous membranes, by muscular and vascular weakness, disturbances of the gastrointestinal tract and nervous system, and final cachexia and death. Anatomically it is accompanied by disease of both adrenals, usually a caseous tuberculosis. We shall not attempt a description of the symptomatology or pathology of the disease.

The clinical diagnosis of outspoken cases of Addison's disease is sometimes easy. If there is a definite history of weakness, vomiting, constipation and diarrhœa, abdominal and lumbar pains and there is present a pigmentation of the skin and mucous membranes, and when pernicious anæmia and a few other conditions which might be confused with it can be excluded, the diagnosis is reasonably certain. In Addison's disease there is a mononucleosis and a hypereosinophilia, in the blood.

Before the pigmentation occurs, however, the diagnosis is extremely difficult or impossible. Latent Addison's disease and other conditions of hypoepinephria

can only be disclosed by the application of principles of functional diagnostic methods. Unfortunately, the principles upon which a functional investigation might be applied, toward the elucidation of adrenal disturbances, have not been as yet developed to an extent where they can be of great practical assistance to the clinician.

One very evident possibility, however, suggests itself. If states of hypoepinephria are accompanied by diminished function of the adrenals, there must be a lessened amount of the substances in the blood which represent the gland's activity. There is no practical way at present to make use of such an hypothesis. Tests for increased amounts of adrenalin in the blood have been used to discover the opposite state of hyperadrenalism and these tests will be described below.

Eppinger, Falta and Rudinger [45] showed that in cases of Addison's disease (hypoadrenalism) the sugar tolerance is remarkably high. Polak [46] was unable to produce a glycosuria with 2 mg. doses of adrenalin in a case of Addison's disease. Similar doses in normal persons invariably produce glycosuria. Meyer and Kahn corroborated these findings. These facts form the basis for the application of various kinds of glycosuria tests to the functional diagnosis of hypoadrenalism.

Tests of Increased Sugar Tolerance as Evidence of Hypoadrenal Function.—The so-called sugar tests have been described at length in the chapter on liver function testing. (See page 17.) In every case of suspected adrenal disease the sugar tolerance should be investigated.

[45] See Erkrankungen der Blutdrüsen. Falta. Wien, 1913.
[46] Wien. klin. Wchnschr., 1909.

2. Hyperfunction of the Suprarenal Glands

Just what morbid conditions are associated with or produced by hyperfunction of the adrenals is by no means clearly understood. Certain tumors of the chromaffin tissues have been held to produce symptoms connected in some way with hyperadrenalism. The question as to whether other conditions besides tumors of the adrenals can give rise to states of hyperfunction is not decided.

A number of pathological states of the organism have at one time or another been claimed to owe their origin to hyperfunction of the suprarenal glands or chromaffin tissues generally. A school of pathologists in France has for a long time endeavored to explain the heightened blood pressure of nephritis on the ground of an increased function of the suprarenal glands. Further than this it has been held that the arteriosclerosis which accompanies the circulatory hypertonia is the result of hyperadrenal function. Finally according to some pathologists the whole process, of which circulatory hypertonia and nephritis form important parts, is to be ascribed to a primary hyperplasia of the chromaffin tissues.

Certain authors have contended that they were able in such conditions to demonstrate by means of the Ehrmann-Meltzer reaction (*v.i.*) the presence of excessive amounts of adrenalin in the blood.

The question as to just what pathological processes and clinical syndromes are to be held related to hyperadrenalism as effect to cause, is a question almost entirely open and undecided at the present time. Further than this the subject of functional diagnosis of

hyperactive states of the adrenal glands has only begun to be developed.

The principal methods suggested are two in number. The first depends upon the generally accepted influence of hyperadrenalism upon the carbohydrate metabolism. There is said to be always a hyperglycemia. Hence the demonstration of an excess of sugar in the blood is one method of diagnosing a hyperepinephria, provided, of course, that other causes of hyperglycemia can be excluded. There is unfortunately no simple method of making the test.

The second class of functional tests for hyperadrenalism depends upon the demonstration of excess of adrenalin in the blood and the production of glycosuria following the injection of adrenalin.

3. Tests for Adrenalin in the Blood as an Evidence of Hyperadrenalism. The Ehrmann-Meltzer Reaction

It has long been known that intravenous injection of adrenalin produces dilatation of the pupil. This fact has been utilized as a test for adrenalin in various fluids, as blood serum, urine, etc.

Meltzer and Auer,[47] Wessely[48] and others have found that when adrenalin is applied to the frog's eye mydriasis is produced.

Ehrmann[49] studied this phenomenon and suggested it as a delicate test for adrenalin. He found that adrenalin acts upon the dilator (sympathetic) fibers of the iris in a strength of 1 to 20,000,000. Dilatation of the pupil of the frog's bulbus oculi immersed in salt solution occurs when excessively minute quantities of

[47] Centralbl. f. Physiol., 1904, XVIII, 316.
[48] Zeitsch. f. Augenh., Aug. 13, 1905.
[49] Archiv f. exper. Path. u. Pharm., 1905, LIII, 96.

adrenalin are present, quantities as small as .000025 mg. Later investigations have shown, however, that other substances in blood serum will produce the same reaction and therefore the practical availability of the reaction as a test for adrenalin in the serum is vitiated.

4. *Adrenalin Glycosuria as a Test of Hyperfunction of the Chromaffin System*

We owe to Blum [50] the discovery that the hypodermic injection of adrenalin will occasionally produce glycosuria. The reducing substance found in the urine has been proved to be glucose and there is always a hyperglycemia (Metzger [51]). The action of adrenalin in thus producing a hyperglycemia is due to its well known stimulating effect upon the sympathetic nervous system (Underhill [52]) acting upon sugar storing organs and causing them to relinquish their supply of dextrose producing substances as glycogen.

Soon after Blum made this discovery the interesting fact was tested and confirmed in many directions. It was discovered that the glycosuria appears after the exhibition of extract of adrenal substance as well as after that of its active principle, adrenalin.

Adrenalin glycosuria appears after comparatively small doses (.01-.1 mg.) and is readily provoked by a subcutaneous injection. The injection of one or two milligrams of adrenalin is followed in half an hour to two hours by a glycosuria lasting three hours. The glycosuria is always accompanied by a hyperglycemia.

[50] Deutsch. Arch. f. klin. Med., 1901, LXXI, 146.
[51] Münch. med. Wchnschr., 1902, 478.
[52] Amer. Jour. Physiol., 1906-07, XVII, 42.

5. *Deviation of Complement in Functional Diagnosis of Suprarenal Disease*

Polito and Corelli [53] have attempted to apply the complement fixation test to the diagnosis of suprarenal gland disease (hyperfunction), using an alcoholic extract of suprarenal gland as antigen. Their results were indeterminate.

V. THE HYPOPHYSIS

The pituitary gland or hypophysis, as is well known, is composed of two portions, a larger anterior epithelial, follicular, glandular portion and a posterior lobe consisting of connective and vascular structures. Between the two is a partly glandular, partly vascular portion, the pars intermedia. The whole organ is contained in a bony inclosure, the sella turcica, or pituitary fossa of the sphenoid bone.

Since Marie first described the disease, acromegaly, and Rogowitch noted hypertrophy of the pituitary after thyroidectomy, both of which took place in 1886, a very large literature has sprung into existence concerning the physiology and pathology of the hypophysis.

The deep situation of this interesting organ at the base of the brain makes experimental investigation very difficult. The embryological and histological differences between the anterior and posterior lobe of the hypophysis, made it extremely probable early in the history of these investigations that a different functional activity must be attributed to the two portions.

[53] La Nouva Riv. Clin. Terap., 1911, XIV, 482.

In this respect there is an analogy with some of the other ductless glands.

The differentiation of the two systems in the hypophysis, from the standpoint of pathology, is especially difficult because of the confined space in which the organ is lodged, making it almost inevitable that disease of one portion will affect the other.

The name of Cushing in our country is intimately associated with our knowledge of the hypophysis on account of his extensive experimental and clinical investigations.[54]

There is still much to be learned concerning the physiology and pathology of the hypophysis. Certain facts, are, however, pretty well agreed upon. The pituitary is probably essential to life, i. e., it is a vital organ. After its removal, animals soon die with severe cachexia. The secretion of the posterior lobe is supposed to gain access to the cerebrospinal fluid and the general circulation. It is concerned in regulating metabolism, particularly that of carbohydrates. It affects also the growth of fat. The internal secretion of the anterior lobe affects the processes of general metabolism and especially growth.

Oliver and Shafer in 1895 [55] discovered that extracts of the pituitary produce when injected into blood vessels, a rise of blood pressure, like that of the adrenals. Three years later Howell [56] discovered that only extracts of the posterior lobe have this effect.

As with the thyroid and adrenals there is the same tendency among clinicians to regard the pathology of the hypophysis as being manifested by states of hyper- and hypo-function. Acromegaly or Marie's

[54] The Pituitary Body and its Disorders, Phila., 1912.
[55] Jour. of Physiol., 1895, 18.
[56] Jour. Exper. Med., 1898, 3.

disease is regarded as a typical example of the former, while adiposo-genital dystrophy or Fröhlich's disease is looked upon as an equally typical example of the latter.

In our very brief account of the functional diagnosis of the pituitaropathies, brief because so little of importance has been accumulated in medical literature, we shall consider the two states, opposite and distinct, producing diametrically opposite effects upon the organism: hyperpituitarism and hypopituitarism.

1. *States of Hyperpituitarism*

The most typical example is acromegaly or Marie's disease, first described by him in 1886.[57]

Acromegaly is a chronic disorder characterized by an abnormal increase in the size of the nose, lips, tongue, lower jaw, hands and feet, by hyperplastic changes in the bones and soft parts, usually accompanied by considerable enlargement of the hypophysis and widening of the sella turcica. Symptoms of increased intracranial pressure often occur. The vegetative nervous system is in a state of hyperirritability. The most common pathological finding is adenoma of the anterior part of the hypophysis. There is increase of function of the glandular hypophysis, a true hyperpituitarism.

We shall not enter into the etiology and pathology or into the detailed account of the general symptomatology of acromegaly. As to the general diagnosis of the disease it may be said to be quite easy in outspoken and typical cases. It is, however, difficult in the early stages, the so-called latent period of the disease. Acromegaly must be differentiated from cer-

[57] Revue de Méd., 1886, p. 298.

tain diseases which may partially resemble it. Brain tumor, arthritis deformans, Graves' disease, diabetes, progressive muscular atrophy, have all been diagnosed as present when acromegaly really existed.[58]

Combinations of acromegaly with Basedowian or myxedematous symptoms sometimes occur in the earlier stages. X-ray examination of the sella turcica (Oppenheim) is, as is now well known, of extreme diagnostic value.

Two methods of functionally determining the existence of hyperpituitarism have been suggested. They are:

1. Demonstration of Disturbed Metabolism, as shown by increase of gas exchange.

2. Demonstration of Alimentary or Spontaneous Glycosuria.

1. Demonstration of Metabolic Disturbance as Shown by Increase of Gas Exchange as an Aid to the Functional Diagnosis of Hyperpituitarism.—Very little work has been done upon the study of the gas exchanges in acromegaly. That which has been done has been carried out with the Zuntz-Geppert apparatus. Cases have been examined by Magnus-Levy, Salomon, Bernstein and Falta.

In all, there are seven cases reported, in which details are given as to the clinical facts and the amount of oxygen consumption and carbon dioxide production in ccm. per kilogram per minute. The figures vary for oxygen from 5.19 ccm. down to 3.55 and for CO_2 from 4.33 down to 2.73. Falta states that the results of the cases so far investigated do not demonstrate an in-

[58] Modern Med., Osler-McCrae, 1915, IV, 813. (Dock.)

evitable increase in gas exchanges in acromegaly, as is the case in hyperthyroidism.

It appears to be the opinion of Magnus-Levy and Salomon that if hyperpituitarism is uncomplicated by disorder of other glands of internal secretion (as thyroid) there is no increase in the gas exchanges. In this opinion Falta [59] concurred.

2. *Demonstration of Spontaneous and Provocative Glycosuria as a Functional Test for Hyperpituitarism.* —It has been known for some time that acromegaly is often accompanied by a temporary or permanent glycosuria. Marie, who first described the disease, called attention to this fact. Borchard, from a study of 176 cases, from the literature found spontaneous glycosuria reported in 63, and alimentary glycosuria in 8. In 8 cases studied by Falta there was spontaneous or alimentary glycosuria in 5. Glycosuria appears only in the early stages of the disease, disappearing towards the end with the beginning of cachexia.

The test for provocative alimentary glycosuria should be made in every case of suspected hyperpituitarism.

It will be unnecessary here to repeat the details of technique of the various tests for provocative glycosuria, since they have been fully dealt with under a previous chapter. The four tests there described are (1) the Cane Sugar Test, (2) the Glucose Test, (3) the Levulose Test, (4) the Galactose Test.

The Glucose test has been more frequently used than the others in testing the carbohydrate powers in cases of suspected hyperpituitarism.

[59] Erkrankungen der Blutdrüsen, Berl., 1913, p. 213.

2. *States of Hypopituitarism*

This state is typically represented in the so-called hypophyseal dystrophy of Fröhlich, or dystrophia adiposo-genitalis.

Fröhlich,[60] in 1901, first emphasized the connection between destructive tumors of the hypophysis and the occurrence of the syndrome which bears his name and whose chief characteristics are a rapidly developing adiposity, infantilism of the genitalia and myxedematous degeneration of the subcutaneous tissues. Many authors have since reported cases.

The opposite conditions with respect to gas exchanges and glycosuria obtain in hypopituitarism as compared with hyperpituitarism. In hypopituitarism, therefore, the same functional tests are applied as were discussed above under acromegaly. The results, however, will be the opposite. The gas exchanges will be diminished and glycosuria, both spontaneous and provocative, will be negative.

VI. THE VEGETATIVE NERVOUS SYSTEM

(*Autonomic and Sympathetic*)

The very close relation which exists between the functional activity of the glands of internal secretion, and the vegetative nervous system, makes the vital integrity of the one depend to a considerable extent upon the vital integrity of the other. For this reason it seems eminently proper to introduce into a work devoted to the subject of vital function testing a description of any methods which may have been developed for testing the physiological status of the vegetative nervous system.

[60] Wien. klin. Rundschau, 1901, XLVII, 48.

1. *Vagotonia and Sympathicotonia*

Much light has been recently thrown upon the relations which exist between disturbances of function of the autonomic and sympathetic portions of the vegetative nervous system and certain fairly definite clinical syndromes. It has become a matter of common medical parlance since the interesting researches of Eppinger and Hess * to speak of two opposite states of physiological activity of the vegetative nervous system, namely, vagotonia and sympathicotonia, both being supposed to be due to an increased tonus or physiological activity of the autonomic and sympathetic nervous system respectively.

It has long been known that anatomically as well as physiologically and pharmacologically, two types of nervous function exist in the vegetative nervous system, namely, the sympathetic and autonomic; fibres composing the former springing from the dorsal and those of the latter from the cephalic and sacral extremities of the cerebrospinal axis.

Various drugs, especially pilocarpine, muscarine, physostigmine, atropine, and adrenaline, have selective actions upon the terminations of the sympathetic and autonomic nerves, and these actions, long known to the pharmacologist, have in a few instances been made the basis for tests to determine their physiological integrity.

Clinically, it has been found that many individuals, according to spontaneous or provoked reactions, may be classified as vagotonics or sympathicotonics according to the type and character of certain symptoms which they show or to their response to drugs or other

* Die Vagotonie, 8°, Berlin, 1910; also Transl. by Kraus and Jelliffe, 8°, N. Y., 1915.

stimuli. Such symptoms may either be evident upon a superficial examination or will be discovered as a result of clinical investigation.

Vagotonia.—Clinically, hypervagotonia is indicated by the presence of some of the following symptoms: paleness of the face, tendency to myopia, bradycardia, low blood pressure, moisture of the skin, asthma, mucous colitis, gastric hypersecretion, rapid gastric motility, spasmodic constipation and pyloric spasm. Besides these manifestations in vagotonics there will be found a characteristic reaction to pilocarpine (see test below). The symptoms will be favorably influenced by atropine, many of them being diminished or removed entirely after the administration of this drug.

Sympathicotonia.—Clinically, these cases present the opposite set of symptoms, especially tachycardia and hypertension. There will be found in sympathicotonics a hypersensitiveness to epinephrin, a relative insusceptibility to pilocarpine and atropine and various symptoms of increased tone throughout the sympathetic system proper.

The only tests which have been practically worked out for determining the functional integrity of the autonomic nervous system are two in number. (1) The oculocardiac reflex test of Ashner, (2) the pilocarpine test. Only one test has been suggested for directly determining the physiological status of the sympathetic portion and that is the so called adrenalin test.

2. *The Oculocardiac Reflex Test of Ashner* †

Ashner in 1908 described this reflex which consists normally in a change of the heart's rate following pressure on one or both eyeballs.

† Wien. klin. Wchnschr, 1908, xxi, 1529.

The path of the reflex is centripetally through the fifth cranial nerve to the medulla and centrifugally through the vagus, or more rarely, the sympathetic nerves.

Test: After having recorded a normal pulse rate for the individual to be examined, close the eyelids and exert gently increasing pressure by means of the ends of the fingers upon the bulbs of the eyes just under the supraorbital ridges (to avoid direct pressure upon the cornea) until the pressure becomes slightly uncomfortable to the patient; then maintain this pressure for one minute, taking the pulse rate at the same time with the aid of an assistant. In hypervagotonics this reflex which normally slows the pulse rate is exaggerated; that is to say, the pulse rate is slowed more than 10 beats to the minute. During the test there may be some pain, flushing of face, movements of deglutition and apnœa, but these symptoms readily subside upon removal of pressure. While in vagotonics the pulse rate is slowed more than ten beats to the minute below the normal rate, in sympathicotonics the reflex will be diminished or absent; in the first case the pulse rate will be increased and in the second it will be unaffected.

3. *Pilocarpine Test*

A hypodermic injection of .01 gm. of pilocarpine is administered. In the hypervagotonic or pilocarpine-sensitive patient there will be salivation, sweating, nausea, epiphora, flushing and a fall in blood pressure.

4. *Adrenalin Test*

A hypodermic injection of .001 gm. of adrenalin is administered. In sympathicotonic or epinephrin-sensitive patients there will be a sensation of cold, with rigor, tremor, glycosuria and a rise in blood pressure.

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